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CHEMICAL COMPOSITION OF AMERICAN-GROWN FRENCH CIDER APPLES AND OTHER APPLES OF LIKE CHARACTER[1]

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INTRODUCTION

One of the investigations in progress in the fruit and vegetable utilization laboratory of the Office of Horticulture has had as its object the devising of better methods for making unfermented apple and grape juices for use as beverages. Very early in the work it was realized that one of the serious difficulties to be overcome in making acceptable beverage apple juices arises from the deficiency in astringent substances characteristic of nearly all dessert and of most of the culinary apples. In order to meet with popular approval, a beverage juice must possess a rather definite relationship or balance between the amounts of sugar, acid, and astringent substances it contains, since judgments of taste or palatability of a liquid are based upon the perception by the nerves of taste of its sweetness, acidity, and astringency. Very few of the American dessert apples and comparatively few of those grown for culinary uses have sufficient quantities of astringent substances, when the condition of cider ripeness has been attained, to make their juices acceptable. As a whole their juices are insipid or acid-sweet, not because of excessive sugar content, but because of deficiency of tannin and in many cases of acid also. In order to make acceptable beverages, such juices must be given the requisite balance between constituents by blending with other juices having larger astringent content. It is relatively easy to find fruit of sufficiently high acidity for use in correcting any deficiency in that respect, but fruits capable of supplying added astringency must be sought outside the range of the varieties generally cultivated in the United States.

The French cider makers have long recognized the necessity for the blending of juices in order to make balanced and palatable fermented ciders, and the French pomological literature classifies the varieties employed for cider making into groups based on their outstanding characters, as "douce," "acide," "amère," "douce-amère," and the like. Among American workers, Alwood (*1*, *2*)[2] and Price and Ellett (*7*) have recognized that apples of such special types are a prerequisite to the making of fermented apple beverages of a type and quality comparable with the French products. That such material is equally

[1] Received for publication July 28, 1926; issued May, 1928. This paper is the fifth in a series of studies on fruit juices.

[2] Reference is made by number (italic) to "Literature cited," p. 406.

indispensable to the making of unfermented beverage juices of the highest quality does not seem to have been recognized.

In the course of the analytical work upon apples carried on during the last seven years, an attempt has been made to obtain as much information as possible in regard to apples of high astringent content, and a considerable number of apples of this character have been analyzed at various times as material and opportunity offered. The analyses include a number of French cider apples ("French crabs"), for some of which it is believed no analyses of material grown in America can be found in the literature, together with a number of crab apples and highly astringent apples of various origins.

MATERIAL AND METHODS

The fruit employed in the analyses was grown in the apple variety collection of the Office of Horticulture, Bureau of Plant Industry, at the Arlington Experiment Farm, Rosslyn, Va., near Washington, D. C. The varietal identifications have been verified by tracing the scions to their sources in all doubtful cases and by repeated checking of the fruit with varietal descriptions. The French cider apples are fairly well represented in the Arlington orchard, many of the trees having been grown directly or indirectly from scions brought from France by Alwood (*1*).

In order to obtain some indication of the degree of modification in character of these apples occurring when grown in widely differing environments, samples of fruit were obtained in 1923 from the collection growing at Blacksburg, Va., on the grounds of the Virginia Agricultural Experiment Station, from Alwood's original stocks, for comparison with fruit grown at the Arlington farm. Through the kindness of H. L. Price and other members of the staff of the Virginia station, the entire crop of the varieties in fruit in 1923 was harvested and shipped to Washington. The trees had been in sod for some years, the grass being cut for hay or pastured. They were top-worked upon standard varieties in 1900, the grafts consequently being 23 years old at the time of sampling. Descriptions of trees and fruits and analyses of the whole fruits as grown at Blacksburg were published in 1909 by Price and Ellett (*7*) at the time the trees were 8 years old. Their analyses, with a single analysis of Moulin-à-Vent by Alwood, Davidson, and Moncure (*2*), made upon the first crop borne by the Blacksburg trees, would appear to be the only analyses of American-grown French apples in the literature.

The general method of handling the material employed in the analyses of the fruit grown at the Arlington farm and the cultural treatment of that orchard have been fully described elsewhere (*4*). The fruit was picked at the stage of picking ripeness and held in a cool basement room until it had reached proper condition for pressing. The samples were tree run, the quantities ranging from 2 to 10 bushels, and the only sorting done was confined to the discarding of partially decayed fruits when such were present. In the case of the material of Bramtot, Launette, and Rouge du Landel, grown at Blacksburg, the fruit was ready for pressing when picked. Shipment by freight to Washington required five or six days, during which the temperature in the car ranged rather high, and the fruit consequently showed considerable decay and was slightly past prime

condition for pressing when received at Washington. It was therefore pressed immediately. With these exceptions, the fruit was all in prime cider-ripe condition when the pressing was done. Care was employed to obtain uniform pressing of the samples, and the analyses were begun immediately. The bulk of the juice obtained was preserved by pasteurization, and a repetition of the analyses after pasteurization has incidentally served as verification of the determinations of total sugar and titratable acidity made upon the fresh juices.

It was necessary to determine for the present purpose what is meant by an astringent apple. In the analyses made in this laboratory, the extremes of total astringency encountered have been 7.4 and 955 mgm. per 100 c. c. It is necessary to draw a line somewhere across this range and to consider everything above it as in the astringent class. This must be done arbitrarily. As culinary varieties rarely equal or exceed 125 mgm. of total astringents per 100 c. c. in juice when expressed at the cider-ripe stage, it is probably fair to fix the arbitrary limit at that figure. The present paper consequently includes not only analyses of French, Siberian, and other crabs, but also of all apples of whatever origin which have been found to have a total astringency consistently equaling or exceeding 125 mgm. per 100 c. c. For the sake of completeness a number of analyses previously published in communications from this laboratory have been included in the tables.

It should be clearly understood that the fact two juices have identical amounts of total astringent materials does not necessarily imply that they are equally astringent to taste, even though their acid and sugar content should also be identical. Total astringency is made up of the two components, tannins and astringent nontannins. Nothing is known as to the relative effects of equal amounts of true tannin and of astringent nontannin upon the nerves of taste, and it is possible, indeed certain, that quite different effects upon the nerves of taste are produced by two juices having the same total astringency but differing in the amounts of tannin and nontannin. Moreover, the term "astringent nontannins" is a collective one, applied to all substances which reduce $KMnO_4$ but are not precipitated by gelatin. The nature of these substances is largely unknown, but it is certain that they are of varied character and that they differ markedly in their effect upon the nerves of taste. The bittersweet group of apples, for example, contain varying amounts of a bitter constituent, probably glucosidal in nature, which is not encountered in apples of other types. The nature as well as the relative amounts of the astringent substances consequently vary from variety to variety, and as a result a determination of the total amount does not give an accurate measure of the astringency of the juice as measured by the sense of taste.

Determinations of total astringency are therefore like determinations of total sugars in which varying and unknown amounts of sucrose, dextrose, levulose, and pentoses are present. Such determinations tell nothing as to the relative sweetness of the mixtures as determined by the sense of taste. In the case of sugars a very accurate idea of the sweetness of a mixture is attainable by determining the amounts of the individual components, but so little is known of the astringent substances of fruits that a similar treatment can not as yet be applied to them.

SEASONAL CONDITIONS IN RELATION TO ANALYTICAL RESULTS

The analyses presented herewith were made in the years 1920 to 1925, inclusive. In a considerable number of cases several analyses were made upon a given variety, either in consecutive years or at intervals throughout the period occupied by the work; in other cases only a single analysis was made. In other investigations which were proceeding concurrently with the present work (*4*, *5*), a detailed study of the climatological conditions of the years 1920 to 1925, inclusive, was made with respect to the effects of the seasonal conditions upon the composition of the fruit of somewhat more than 200 varieties of apples. As a result of this investigation it was found that the seasonal conditions of the growing period determine the composition of the crop of the year, all the varieties employed behaving in very high degree as a unit. In consequence, the six years covered by the study can be ranked in order with respect to the sugar content of the fruit produced. Acid and astringency content of the fruit is correlated with sugar content in a very definite manner, the correlations being consistently maintained throughout the period of the work.

From the results of these investigations it appears that of the six years, 1923 was that characterized in a large majority of varieties by the maximum sugar content, maximum sucrose content, next to maximum acid content, and minimum astringency content found in the fruit during the six-year period. The year 1921 stood next to 1923 in point of sugar content of the crop, 1925 third, 1922 fourth, 1920 fifth, and 1924 sixth and lowest. With the minimum sugar content found in 1924 there was associated minimum or next to minimum sucrose and acidity content and maximum astringency. As these results with respect to composition of the fruit of a large group of varieties have been shown to be definitely related to the seasonal conditions of the various years, and as much of the fruit employed in the present work was grown in the Arlington orchard, subject to the same conditions, it may be assumed that these analyses reflect seasonal conditions in the same manner and to the same degree as do those presented in the climatological study. Hence analyses made in 1923 or 1921 represent the composition of the crop as grown under exceptionally favorable seasonal conditions for accumulation of high sugar content and attainment of high quality for the variety, those made in 1924 or 1920 represent the opposite extreme of highly unfavorable conditions for storage of sugar, and those made in 1922 or 1925 an intermediate condition. An isolated analysis in any particular year should be evaluated in the light of these statements, which are based upon evidence presented in detail in the papers to which reference has previously been made (*4*, *5*).

FRENCH CIDER APPLES

Analytical data upon 21 varieties of French cider apples are presented in Table 1. In the case of 5 varieties material grown at both Blacksburg and the Arlington farm was used; in the others fruit from only one location was employed. The material grown at Blacksburg was analyzed in 1923 only, while some of the varieties grown at the Arlington farm were analyzed in two, three, or four

TABLE 1.—*Analyses of apples of French cider varieties grown at the Arlington Experiment Farm, Rosslyn, Va., and at Blacksburg, Va.*

Variety (apples were grown at Arlington farm except as indicated in footnotes)	Date picked	Date analyzed	Constituents (per cent)								Acid-astringency-sugar ratio
			Reducing sugar	Sucrose as invert sugar	Total sugar	Acid	Total astringency	Tannin	Non-tannin astringency	Total solids	
Amère du Surville	Sept. 23, 1922	Dec. 14	8.98	0.94	9.92	0.410	0.3384	0.2136	0.1248	13.91	1:0.82:24.2
	Sept. 4, 1923	Sept. 19	9.70	1.64	11.34	.248	.7400	.4220	.3180	13.21	1:2.98:45.8
	Sept. 1, 1923[a]	Sept. 11	9.58	1.01	10.59	.231	.8050	.5880	.2170		1:3.47:45.8
	Oct. 18, 1924	Nov. 8	8.97	2.01	10.98	.148	.4360	.2795	.1565		1:2.94:74.2
Barbarie [a]	Oct. 10, 1925	Oct. 17	11.06	1.82	12.88	.176	.4740	.2140	.2600	15.87	1:2.69:73.2
Bedan des Parts [a]	Sept. 7, 1923	Oct. 21	10.10	1.54	11.64	.215	.3132	.1699	.1433	13.69	1:1.378:54.1
Bonne de Freuilles	Aug. 28, 1923	Sept. 7	9.14	1.37	10.51	.146	.4070	.2745	.1325	11.40	1:2.75:54.1
	Sept. 2, 1923	Sept. 19	7.73	3.61	11.34	.330	.3280	.2050	.1230	13.83	1:0.99:34.3
Bramtot	Sept. 11, 1922	Sept. 24	11.94	3.69	15.63	.316	.3385	.1735	.1650		1:1.07:49.4
	Sept. 6, 1923	Sept. 19	11.84	4.10	15.94	.187	.4600	.2432	.2168	17.82	1:2.46:85.2
D'Avrolles [a]	Sept. 2, 1923[a]	Sept. 7	10.32	.69	11.01	.343	.6100	.4010	.2090	12.93	1:1.77:32.1
Fréquin Lajoie [a]	Oct. 4, 1923	Oct. 18	11.53	1.83	13.36	.210	.4148	.2991	.1157	14.68	1:1.97:63.6
Fréquin Rouge	Aug. 27, 1923	Sept. 10	7.49	2.89	10.38	.318	.2780	.1515	.1265	11.56	1:0.87:32.3
	Sept. 16, 1923	Sept. 29	9.71	4.05	13.76	.370	.1568	.0568	.1000	15.58	1:0.42:37.2
Hilaire	Sept. 28, 1922	Dec. 8	9.70	1.34	11.04	.817	.1474	.0677	.0797	14.47	1:0.18:13.5
	Sept. 8, 1923	Oct. 9	9.13	2.71	11.84	.890	.1045	.0393	.0652	15.18	1:0.12:13.3
	Sept. 23, 1924	Oct. 4	8.53	2.13	10.66	.799	.1355	.0798	.0557		1:0.17:13.3
Jouveaux [a]	Sept. 25, 1925	Oct. 29	8.70	1.54	10.24	.437	.0912	.0401	.0511	12.94	1:0.21:23.4
	Sept. 2, 1923	Sept. 7	9.70	1.44	11.14	.178	.3480	.2215	.1265	14.10	1:1.95:62.5
Julian de Paulmier	Sept. 3, 1923	Sept. 19	9.52	3.38	12.90	.350	.6100	.3200	.2900	15.61	1:1.74:37
	Sept. 7, 1924	Sept. 18	8.35	3.37	11.72	.185	.4720	.1580	.3140		1:2.55:63.3
	Oct. 5, 1925	Oct. 12	8.80	3.52	12.32	.182	.3260	.0860	.2400	15.09	1:1.79:67.6
Launette	Sept. 1, 1923[a]	Sept. 7	8.55	3.08	11.63	.229	.5025	.2865	.2160		1:2.19:50.8
	Sept. 15, 1924	Sept. 24	9.43	1.21	10.64	.219	.4700	.2450	.2250	11.93	1:2.14:44
Launette Grosse	Sept. 11, 1923	Sept. 18	11.98	3.18	15.16	.229	.6865	.3085	.3780	17.88	1:2.29:50.7
Moulin-à-Vent	Sept. 1, 1923	Sept. 7	7.46	2.31	9.77	.163	.4050	.2330	.1720	11.41	1:2.48:6
	1903[b]		7.27	3.73	11.00	.210	.1830			15.77	1:0.87:52.4
Omont	Sept. 11, 1923	Sept. 19	12.17	3.45	15.62	.256	.6400	.3290	.3110	21.13	1:2.5:61
Passe Reine	Sept. 7, 1924	Sept. 18	8.50	1.87	10.37	.164	.2680	.1060	.1620		1:1.63:95
Petite Douce Rousse	July 13, 1922[b]	July 28	7.86	1.61	9.47	.988	.2984	.1339	.1645	11.20	1:0.3:9.58
	Sept. 16, 1924	Sept. 24	8.20	3.32	11.52	.202	.4110	.2628	.1482	14.36	1:2.03:57
Précoce de Tunis	July 21, 1920	Aug. 12	7.80	.54	8.34	.192	.2239	.0714	.1525		1:1.16:43.5
	July 13, 1922	July 18	6.24	1.89	8.13	.348	.1635	.0716	.0919	9.51	1:0.47:23.3
	July 16, 1923	July 26	7.76	3.87	11.63	.358	.3910	.1900	.2010	13.92	1:1.09:32.5
Reine des Pommes	Sept. 17, 1923	Sept. 22	8.15	3.18	11.33	.189	.4440	.2855	.1585	14.05	1:2.34:60
Rossignol [a]	Sept. 1, 1923[a]	Sept. 7	8.80	3.10	11.90	.329	.5945	.3965	.1980	13.80	1:1.8:36
	Sept. 16, 1922	Oct. 21	10.23	1.99	12.22	.332	.1982	.0956	.1026	15.37	1:0.6:36.8
Rouge du Landel	Sept. 1, 1923[a]	Sept. 8	8.20	1.80	10.00	.355	.2920	.1452	.1468	11.76	1:0.823:28.2
	Sept. 7, 1923	Sept. 20	8.23	1.45	9.68	.296	.2512	.1002	.1510	11.93	1:0.848:32.7
	Sept. 7, 1924	Sept. 15	7.58	2.91	10.49	.171	.2920	.1575	.1345		1:1.7:61.3

[a] Material from Blacksburg, Va.

[b] Analysis at Blacksburg, Va., quoted from Alwood, Davidson, and Moncure (*1*).

years. Reports of analyses of the whole fruit of some of these varieties have been made by Price and Ellett (*7*). Analyses of material grown in France have been compiled by Power (*6*), by Truelle (*8*), and by Warcollier (*9*). For purposes of comparison, some of the results given by these authors are reproduced in Table 2. For the sake of completeness, data have been included for a few varieties analyzed by the French workers and by Price and Ellett which were not available for the present work.

TABLE 2.—*Summary of analyses of juices and fruit of French-grown French cider apples and of French cider apples grown at Blacksburg, Va., and at the Arlington Experiment Farm, Rosslyn, Va.*

Variety and product [a]	Total sugar	Acidity as malic [b]	Total astringency	Solids
	Per cent	*Per cent*	*Per cent*	*Per cent*
Amère du Surville:				
Juice (French)	14.19–15.21	0.158–0.250	0.300–0.807	
Juice (Arlington)	9.92–12.88	.148– .210	.338– .740	13.91–15.87
Juice (Blacksburg)	10.59	.231	.805	13.21
Whole fruit (French)	12.20	.350	.0760	20.2
Whole fruit (Blacksburg)	9.48–12.40	.464– .778	.570– .760	19.92–23.65
Bedan des Parts:				
Juice (French)	14.02–16.32	.191– .240	.152– .310	
Juice (Blacksburg)	10.51	.146	.407	11.40
Whole fruit (French)	12.61	.360	.170	21.60
Whole fruit (Blacksburg)	11.68	.601	.360	21.58
Binet Blanc:				
Juice (French)	17.14	.220	.190	
Whole fruit (French)	16.48	.380	.150	19.70
Whole fruit (Blacksburg)	12.38	.396	.130	19.78
Binet Rouge:				
Juice (French)	15.30–17.60	.125– .24	.191– .270	
Whole fruit (French)	11.93	.25	.160	20.5
Whole fruit (Blacksburg)	14.12	.546	.240	27.80
Bonne de Freuilles:				
Juice (Arlington)	11.34	.330	.328	13.83
Whole fruit (Blacksburg)	14.52	.560	.510	21.65
Bramtot:				
Juice (French)	16.00–16.95	.215– .326	.287– .523	
Juice (Blacksburg)	11.01	.343	.610	12.93
Juice (Arlington)	15.63–15.94	.187– .316	.338– .460	17.82
Whole fruit (Blacksburg)	14.52	.560	.510	21.65
Julian de Paulmier:				
Juice (French)	16.90	.190	.330	
Juice (Arlington)	11.72–12.90	.182– .350	.326– .610	15.09–15.61
Whole fruit (French)	11.86	.330	.160	22.00
Whole fruit (Blacksburg)	16.54	.218	.380	26.34
Launette Grosse:				
Juice (French)	16.07–16.80	.196– .230	.517– .650	
Juice (Arlington)	15.16	.229	.686	17.88
Whole fruit (French)	14.52	.270	.550	21.50
Whole fruit (Blacksburg)	10.10–10.70	.415– .574	.230– .480	19.80–20
Maréchal:				
Juice (French)	16.80	.260	.970	
Whole fruit (French)	13.50	.330	.500	22.2
Whole fruit (Blacksburg)	12.85	.410	.500	22.4
Michelin:				
Juice (French)	15.92–16.40	.168– .180	.210– .430	
Whole fruit (French)	10.92	.32	.220	19.30
Whole fruit (Blacksburg)	13.05	.725	.290	23.92
Montlige Blanc:				
Juice (French)	15.87	.200	.180	
Whole fruit (French)	11.60	.290	.210	19.80
Whole fruit (Blacksburg)	[c] 6.43	.164	.160	17.17
Moulin-à-Vent:				
Juice (French)	16.57–18.00	.198– .290	.350– .365	
Juice (Blacksburg) [c]	11.00	.210	.183	15.77
Juice (Arlington)	9.77	.163	.405	11.41
Whole fruit (French)	12.37	.60	[c] .040	21.10
Whole fruit (Blacksburg)	11.27	.410	.410	20.65

[a] Analyses of juices or whole fruits designated as "French" are quoted from Power (*6*), Truelle (*8*), or Warcollier (*9*), and are usually averages of 3 to 10 analyses. When two values are presented, they are extremes given by two or more of these authorities. Analyses of juices designated as "Arlington" or "Blacksburg" were made by the writer upon fruit grown at the location indicated. Analyses of whole fruit designated as "Blacksburg" were made by Price and Ellett (*7*).

[b] The values for acidity have been recalculated where necessary and are expressed as malic.

[c] Analysis of fruit and juice at Blacksburg by Alwood, Davidson, and Moncure (*2*).

TABLE 2.—*Summary of analyses of juices and fruit of French-grown French cider apples and of French cider apples grown at Blacksburg, Va., and at the Arlington Experiment Farm, Rosslyn, Va.*—Continued

Variety and product	Total sugar	Acidity as malic	Total astringency	Solids
Omont:	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Juice (French)	14.20	0.33	0.260	
Juice (Arlington)	10.37–15.62	0.164–.256	0.268–.640	21.13
Whole fruit (French)	12.34	.37	.200	20.6
Whole fruit (Blacksburg)	13.60	.232	.210	22.26
Passe Reine:				
Juice (Arlington)	9.47	.988	.2984	11.20
Whole fruit (Blacksburg)	13.02	.464	.760	22.55
Petite Douce Rousse:				
Juice (French)	15.56	.270	.180	
Juice (Arlington)	11.52	.202	.411	14.36
Whole fruit (French)	11.30	.410	.140	21.00
Whole fruit (Blacksburg)	9.65	.505	.470	[4] 29.80
Reine des Pommes:				
Juice (French)	16.73–19.10	.184–.430	.268–.510	
Juice (Arlington)	11.33	.189	.444	14.05
Juice (Blacksburg)	11.90	.329	.594	13.80
Whole fruit (French)	12.96	.570	.240	19.90
Whole fruit (Blacksburg)	10.62–13.20	.428–.847	.340–.540	19.75–26.3
Rouge du Landel:				
Juice (French)	14.12–15.07	.242–.230	.210–.283	
Juice (Blacksburg)	10.00	.355	.2920	11.76
Juice (Arlington)	9.68–10.49	.171–.296	.251–.292	11.93
Whole fruit (French)	12.20	.31	.120	19.70
Whole fruit (Blacksburg)	8.09	.53	.200	17.55
St. Laurent:				
Juice (French)	15.50–15.90	.121–.340	.152–.340	
Whole fruit (French)	13.88	.21	.210	25.50
Whole fruit (Blacksburg)	16.04	.314	.270	28.30

[4] So in original; probably a typographical error.

The data thus assembled afford material for formulating an answer to the most important question with respect to the French cider apples, namely, whether the transfer from France to America has resulted in such fundamental changes in composition as to destroy their distinctive character as a group. This question may be definitely answered in the negative. As compared with the analyses of the same variety grown in France, the analyses of the fruit grown at Blacksburg or at Arlington show no large or consistent differences. The values for total sugar given by the French workers for juices are in practically every case somewhat higher than those found in the American work. The practically universal use of the saccharimeter instead of chemical determinations in the French studies of juices undoubtedly is responsible for the high values shown. It has repeatedly been demonstrated that the use of a saccharimeter with juices having a high content of nonsugar solids gives values materially in excess of those found by chemical determinations (*3*). That this is the real explanation of the high sugar content of French juices is made conclusive by the fact that the French determinations of sugar in the whole fruit, which must be made by chemical methods, agree quite well with the data on American-grown whole fruit. The disagreement between the French data on sugar in the whole fruit and in the juice of the variety is conclusive evidence that the juice figures are in excess of the true values.

In acid content the determinations upon French and American material, and particularly upon juices, show in general very close agreement. In tannin content the differences between the two

materials are greater, the American samples showing amounts sometimes larger, sometimes smaller than French fruit of the same variety, so that the differences do not indicate change in a definite direction. The differences between the samples grown at Arlington and at Blacksburg are in some cases greater than those between either and the samples grown in France.

Warcollier (*9*) and Truelle (*8*) collected data upon maximum, minimum, and mean composition of a number of French cider apples, based upon considerable numbers of analyses from various sources and from various regions of France. These show very wide ranges in amount of all constituents, as will be noted by reference to the examples reproduced from Warcollier in Table 3. There is a range of 100 per cent in sugar content in these analyses and of several times this amount in acid and tannin when the numbers of analyses are such as to suggest that the various soils and climatic conditions of France are adequately represented. The differences in composition of American-grown fruit from the French material analyzed by Power, Truelle, and Warcollier, as shown in Table 2, lie well within the extremes found in material from various parts of France.

TABLE 3.—*Maximum, minimum, and mean composition of juices of standard sorts of French cider apples*

[From Warcollier (*9, p. 393*)]

Variety	Number of analyses	Item	Total sugar	Acid	Tannin
			Per cent	*Per cent*	*Per cent*
St. Laurent	21	Maximum	24.31	0.730	0.699
		Minimum	12.63	.090	.096
		Mean	16.51	.276	.244
Bramtot	58	Maximum	26.35	.960	1.055
		Minimum	9.41	.085	.133
		Mean	19.05	.219	.529
Omont	3	Maximum	14.92	.370	.300
		Minimum	12.90	.310	.245
		Mean	14.19	.330	.266
Bedan des Parts	75	Maximum	21.94	.397	.825
		Minimum	10.68	.015	.008
		Mean	14.89	.140	.196

It may therefore be taken as rather definitely established that the French cider fruits, grown in Virginia at an elevation of 2,170 feet, as at Blacksburg, or only a few feet above sea level, as at Arlington, retain their distinctive characters without modification in any definite direction. All the apples here dealt with, with the exception of Passe Reine and Saint Hilaire, belong to the group called by the French "douce-amère" and by the English "bitter-sweet." They are characterized by a very high content of astringent materials and by an acidity lower than that of most of the American dessert varieties. The sugar content is not markedly higher than that of the better American dessert fruits, but is made to appear so to taste by the low acidity, while the high astringency and the persistent bitter aftertaste give them a very distinctive character.

It is believed that the combination of low acidity, high astringency, and medium to high sugar content found in these apples renders them potentially valuable material for blending with fruits of the dessert and culinary types in the making of unfermented juices and possibly

of other products. As is evident from their chemical composition, nearly all of the American cultivated apples are too low in astringency to furnish a juice of proper balance in its acid-astringency-sugar ratio. Winesap and Northern Spy are varieties which make acceptable beverage juices, primarily for the reason that their astringency at the proper pressing season is much higher than that of other commercial sorts. But it is not sufficiently high to permit extensive mixture of other sorts with them in cider making without upsetting the balance of the product in the direction of insipidity.

For use in blending with the usual types of apples for juice making, an ideal fruit would be one which could be added without disturbing the general level of sugar or acid content while bringing astringency up to the desired point.

The French cider apples thus far examined appear to be almost ideal material for meeting these requirements, in so far as the chemical characters of the fruit are concerned. It would seem a justifiable proceeding to give somewhat greater attention to these varieties to the end that their adaptability to varied conditions, relative susceptibility to the more important American diseases and pests, productiveness, and other pertinent horticultural data might be known. Data on the behavior of these apples such as Price and Ellett have given for the Blacksburg collection should be procured in other fruit-growing districts, since it would appear that some of these apples may have a definite place in American fruit growing.

SIBERIAN AND NATIVE CRABS AND OTHER ASTRINGENT APPLES

Table 4 presents analyses of 61 varieties of apples of widely varying origin and character, alike only in that they have a total content of astringent constituents equaling or exceeding 125 milligrams per 100 cubic centimeters. These may be roughly divided into groups on the basis of likeness with respect to other characters.

TABLE 4.—*Analyses of astringent apples of various origins*

Variety	Date picked	Date analyzed	Constituents (per cent)								
			Reducing sugar	Sucrose	Total sugar	Acid	Total astringency	Tannin	Nontannin	Solids	Acid-astringency-sugar ratio
Algérienne	Aug. 19, 1922	Aug. 28	5.93	4.07	10.00	0.383	0.2761	0.1104	0.1657	13.60	1:0.72:27.6
	Sept. 13, 1923	Sept. 21	5.97	5.58	11.55	.221	.2500	.1315	.1185	12.97	1:1.13:52.2
	Aug. 16, 1924	Aug. 24	5.02	4.24	9.26	.231	.1930	.1008	.0922		1:0.83:40
Arctic	Oct. 7, 1925	Oct. 13	8.28	3.66	11.94	.150	.1570	.0812	.0758	14.29	1:1.04:79.6
	Sept. 28, 1922	Dec. 8	8.26	2.15	10.41	.439	.1155	.0358	.0797		1:0.26:23.7
	Sept. 16, 1923	Nov. 15	9.13	4.84	13.97	.608	.1648	.0622	.1026	16.76	1:0.27:23
	Sept. 21, 1924	Oct. 2	6.72	3.38	10.10	.525	.1660	.0760	.0900		1:0.31:19.2
Augustine	Sept. 10, 1925	Sept. 26	8.62	1.84	10.46	.360	.1265	.0518	.0747	13.33	1:0.35:29
	Sept. 3, 1923	Sept. 18	10.42	3.82	14.24	.725	.2182	.0999	.1183	16.09	1:0.3:19.6
Benoni	July 19, 1922	Aug. 9	6.04	2.80	8.84	.426	.1308	.0429	.0879	9.62	1:0.31:20.7
	July 30, 1923	July 30	6.04	4.30	10.34	.655	.1485	.0700	.0785	11.62	1:0.23:15.7
Canada Reinette	Sept. 23, 1922	Dec. 5	8.80	2.94	11.74	.634	.1219	.0701	.0518	15.50	1:0.19:18.5
	Sept. 27, 1923	Oct. 4	7.38	4.79	12.17	1.010	.1270	.0445	.0825	16.69	1:0.126:12
	Sept. 15, 1924	Oct. 6	7.10	3.08	10.18	.710	.1515	.0805	.0710		1:0.21:14.3
Colvert	Aug. 11, 1922	Aug. 14	8.46	2.39	10.85	.769	.1553	.0552	.1001	14.63	1:0.2:14.1
	Sept. 16, 1923	Nov. 22	6.08	3.16	9.24	.425	.1455	.0685	.0770	11.16	1:0.34:21.7
	Sept. 15, 1924	Sept. 19	6.49	2.83	9.32	.494	.1082	.0392	.0690		1:0.22:18.8
Cross	Aug. 12, 1922	Aug. 15	7.82	1.20	9.02	.515	.1482	.0665	.0817	12.55	1:0.287:18.5
	Sept. 8, 1923	Sept. 19	8.26	2.57	10.83	.346	.1485	.0645	.0840	13.35	1:0.43:31.3
Early Joe	Aug. 29, 1924	Sept. 10	7.49	2.04	9.53	.439	.1353	.0798	.0555		1:0.31:21.7
Early Strawberry	July 20, 1922	July 21	6.41	2.19	8.60	.424	.1390	.0583	.0807	9.72	1:0.33:20.2
	Aug. 10, 1924	Aug. 11	7.44	.72	8.16	.352	.1742	.0792	.0950		1:0.49:23.1
Entz	July 25, 1923	July 30	6.18	3.18	9.36	.733	.1375	.0735	.0640	11.20	1:0.187:12.7
	Sept. 18, 1922	Nov. 16	7.26	2.46	9.72	1.117	.3460	.2313	.1147	16.04	1:0.3:8.7
	Sept. 5, 1923	Sept. 18	5.26	2.94	8.20	1.425	.2830	.1347	.1483	11.90	1:0.2:5.75
Florence	Sept. 26, 1924	Oct. 14	6.06	3.21	9.27	1.375	.4130	.2010	.2120		1:0.3:6.7
	July 24, 1922	July 28	6.29	1.19	7.48	.836	.3229	.1441	.1788	9.30	1:0.38:8.9
	Aug. 9, 1924	Aug. 27	7.06	.59	7.65	.639	.2900	.1404	.1496		1:0.47:11.9
Gladstone	July 29, 1925	Aug. 18	8.52	.60	9.12	.620	.1940	.0942	.0998		1:0.31:14.7
	July 20, 1923	July 26	4.87	1.97	6.84	.612	.1410	.0597	.0813	7.62	1:0.23:11.2
	Aug. 5, 1924	Aug. 8	7.22	1.12	8.34	.733	.1930	.0857	.1073		1:0.263:11.3
Golden Sweet	July 31, 1920	Aug. 6	8.86	1.12	9.98	.144	.1280	.0460	.0820	11.40	
	July 25, 1922	July 26	8.37	1.20	9.57	.276	.1768	.0521	.1247		1:0.64:34.6
Grand Sultan	Aug. 2, 1923	Aug. 6	6.09	3.43	9.52	.200	.1880	.0845	.1035	10.72	1:0.94:47.6
	July 3, 1922	July 11	6.61	2.40	9.01	.979	.1327	.0565	.0762	7.62	1:0.13:9.2
Hewes	July 6, 1923	July 13	5.86	1.05	6.91	.894	.1321	.0520	.0801		1:0.14:7.7
	Sept. 3, 1923	Sept. 18	4.98	2.32	7.30	1.050	.1695	.1045	.0650	8.33	1:0.6:6.95

Hort. No. 4951 *	Sept. 24, 1920	Oct. 16	7.72	1.11	8.83	.960	.2660	.1470	.1190	11.20	1:0.27:9.1
	Sept. 16, 1921	Oct. 27	7.39	2.75	10.14	.901	.2790	.1340	.1450		1:0.3:11.2
	Oct. 10, 1922	Nov. 17	7.46	.63	8.09	.915	.2924	.1490	.1434		1:0.32:8.8
	Oct. 24, 1923	Nov. 8	7.33	2.16	9.49	.862	.2838	.1613	.1225	11.62	1:0.33:11
	Oct. 4, 1924	Oct. 16	6.37	2.14	8.51	1.140	.3910	.1815	.2095		1:0.34:7.4
Hogg	Aug. 3, 1922	Aug. 4	6.80	2.71	9.51	1.454	.2023	.0828	.1195	13.14	1:0.14:6.5
	Aug. 14, 1923	Aug. 21	8.30	5.44	13.74	2.259	.1560	.0743	.0817	14.99	1:0.069:6.1
	Sept. 2, 1924	Sept. 15	6.11	3.93	10.04	1.066	.1262	.0690	.0572		1:0.118:9.4
Hog Island	Aug. 3, 1922	Aug. 4	8.91	1.19	10.10	.212	.1921	.0460	.1461	12.76	1:0.9:47.6
	Aug. 27, 1923	Sept. 4	8.20	3.37	11.57	.177	.1261	.0533	.0728	12.48	1:0.7:65.3
Hyslop	Aug. 29, 1922	Sept. 13	5.54	3.48	9.02	.583	.3284	.2077	.1207	11.62	1:0.56:15.4
	Sept. 8, 1923	Sept. 18	4.38	7.03	11.41	.517	.3180	.1768	.1412	12.79	1:0.61:22
	Sept. 13, 1924	Sept. 22	3.37	6.21	9.58	.569	.3700	.2390	.1310		1:0.65:16.8
Irish Peach	Sept. 5, 1925	Sept. 14	3.44	5.92	9.36	.358	.2180	.1345	.0835	7.62	1:0.23:11.2
	July 14, 1922	July 17	5.80	1.93	7.73	.520	.1390	.0511	.0879	11.10	1:0.27:14.9
July	July 10, 1923	July 18	5.85	1.30	7.15	.552	.2750	.1210	.1540		1:0.5:12.9
Jumbo	July 20, 1922	July 25	6.48	1.68	8.16	.869	.1829	.0562	.1267	11.36	1:0.21:9.4
	July 23, 1922	July 25	6.48	4.13	10.61	.852	.1645	.0777	.0868	11.82	1:0.19:12.4
Kennedy	Aug. 2, 1923	Aug. 3	7.26	2.66	9.92	.880	.1540	.0745	.0795	11.99	1:0.175:11.3
	July 21, 1923	July 30	5.49	3.92	9.41	.568	.1368	.0659	.0709	10.80	1:0.24:16.5
Kentucky Sweet	Aug. 23, 1922	Aug. 28	8.89	1.00	9.89	.269	.2002	.1243	.0759	13.59	1:0.74:36.7
	Aug. 27, 1923	Sept. 12	8.31	3.33	11.64	.179	.1960	.0960	.1000	12.63	1:1.09:65
	Sept. 13, 1924	Sept. 27	7.66	2.38	10.04	.154	.1275	.0555	.0720		1:0.82:65.2
Lowland Raspberry	Sept. 1, 1925	Sept. 3	6.74	2.14	8.88	.149	.1180	.0580	.0600	12.54	1:0.78:59.6
	July 16, 1923	July 23	8.08	3.62	11.70	1.010	.2100	.1340	.0760	12.21	1:0.21:11.5
Lubsk Reinette	Aug. 5, 1924	Aug. 8	7.80	.61	8.41	.682	.2000	.0735	.1265		1:0.29:12.3
	July 13, 1920	July 19	7.55	1.47	9.02	.533	.1432	.0594	.0838		1:0.26:16.9
Lyman	July 10, 1923	...do...	6.02	2.74	8.76	.607	.1500	.0568	.0932		1:0.24:14.4
	Aug. 19, 1922	Aug. 29	9.47	2.11	11.58	.685	.5229	.2105	.3124	15.88	1:0.76:16.9
	Sept. 13, 1924	Sept. 22	8.61	3.29	11.90	.511	.4340	.2260	.2080		1:0.84:23.2
Martha	Aug. 26, 1925	Sept. 3	9.98	2.48	12.46	.540	.5200	.2935	.2265	17.67	1:0.96:23
	July 28, 1922	July 29	7.37	2.47	9.84	.936	.1931	.0869	.1062	11.08	1:0.2:10.5
	Aug. 14, 1923	Aug. 27	9.36	2.33	11.69	.850	.2320	.1293	.1027	14.41	1:0.26:13.7
Muster	Aug. 6, 1925	Aug. 10	8.76	2.92	11.68	.824	.1940	.0925	.1015		1:0.23:14.2
	Aug. 15, 1922	Aug. 16	8.92	2.10	11.02	.365	.1246	.0388	.0858	12.86	1:0.34:30.2
Nain de Mahon	Aug. 13, 1923	Aug. 21	8.13	3.04	11.17	.390	.1310	.0685	.0625	13.09	1:0.33:28.6
	Sept. 5, 1924	Sept. 15	7.30	3.03	10.33	.217	.1452	.1025	.0427		1:0.67:47.6
Nain Paradis	Sept. 13, 1923	Sept. 27	7.68	4.32	12.00	.360	.1322	.0373	.0949	14.36	1:0.36:33.3
	Sept. 15, 1924	Nov. 4	8.78	6.58	15.36	.172	.2050	.1020	.1030		1:1.19:89.3
New England Pigeon	July 20, 1922	Nov. 2	7.16	2.94	10.10	.304	.1637	.0796	.0841	11.12	1:0.53:33.2
	July 31, 1923	Oct. 15	8.30	2.99	11.29	.615	.1365	.0643	.0722		1:0.22:18.3
	Aug. 19, 1922	Aug. 25	7.12	2.38	9.50	.395	.1451	.0542	.0909	12.09	1:0.367:24.1
Northern Spy	Sept. 8, 1923	Sept. 23	7.46	1.53	8.99	.212	.1525	.0553	.0972	10.42	1:0.72:42.4
	Sept. 28, 1922	Nov. 3	8.12	2.86	10.98	.393	.1239	.0425	.0814	13.09	1:0.315:27.8
	Aug. 29, 1924	Oct. 8	6.54	3.16	9.70	.567	.1055	.0403	.0652		1:0.18:17.1
Peron	Sept. 16, 1925	Sept. 22	8.06	3.32	11.38	.495	.1340	.0656	.0684	13.61	1:0.27:23.1
	Aug. 16, 1922	Aug. 18	6.36	4.42	10.78	1.055	.1921	.0899	.1022	12.48	1:0.18:10.2
	Aug. 23, 1923	Sept. 5	6.12	4.12	10.24	.580	.1012	.0581	.0631	11.51	1:0.21:17.6
	Sept. 5, 1924	Sept. 16	5.16	3.32	8.48	.458	.1260	.0860	.0400		1:0.27:18.5
	Sept. 1, 1925	Sept. 8	5.56	4.06	9.62	.313	.1415	.0663	.0752	12.43	1:0.45:30.7

* The apple designated as Hort. No. 4951 was believed at the time of planting to be Golden (Golden Beauty). Its identity is in doubt, as it differs in some respects from the descriptions of that variety.

TABLE 4.—*Analyses of astringent apples of various origins*—Continued

Variety	Date picked	Date analyzed	Constituents (per cent)								
			Reducing sugar	Sucrose	Total sugar	Acid	Total astringency	Tannin	Nontannin	Solids	Acid-astringency-sugar ratio
Piper	June 27, 1922	June 30	8.85	0.50	9.35	0.564	0.1614	0.0408	0.1206	10.67	1:0.28:16.5
	July 10, 1923	July 30	6.40	2.40	8.80	.580	.2180	.1010	.1170	10.46	1:0.37:15.1
	July 23, 1924	...do....	5.86	1.02	6.88	.353	.1370	.0545	.0825		1:0.38:19.5
Primate	July 20, 1922	July 21	6.22	3.52	9.74	.478	.1287	.0310	.0977	10.94	1:0.27:20.4
	July 20, 1923	July 23	7.34	2.58	9.92	.890	.1424	.0887	.0537	11.32	1:0.16:11.1
	Aug. 6, 1924	Aug. 11	7.26	.38	7.64	1.385	.0740	.0318	.0422		1:0.053:5.5
Pyrus angustifolia	Oct. 4, 1922	Oct. 4	2.98	.24	3.22	2.316	.6882	.3580	.3302	7.77	1:0.29:1.39
	Oct. 26, 1923	Nov. 23	3.94	1.61	5.55	2.004	.9350	.4960	.4390	10.40	1:0.46:2.7
	Nov. 1, 1924	Nov. 1	2.97	1.47	4.44	2.504	.6900	.3270	.3630	7.63	1:0.27:1.77
	Nov. 4, 1925	Nov. 21	2.94	1.40	4.34	2.568	.8350	.2930	.5420	8.13	1:0.32:1.5
Ramsdell Sweet	Sept. 1, 1922	Sept. 5	9.03	2.15	11.18	.253	.1320	.0388	.0932	13.25	1:0.52:44.2
	Sept. 13, 1924	Sept. 24	8.08	2.78	10.86	.118	.1605	.0575	.1030		1:1.36:92
	Oct. 7, 1925	Oct. 16	8.12	1.78	9.90	.078	.1460	.0475	.0985	12.10	1:1.87:127
Red Astrachan	June 26, 1922	June 30	6.82	2.76	9.58	.960	.2095	.0787	.1308	11.63	1:0.21:9.9
Red Eiser	July 14, 1923	July 18	7.46	3.21	10.67	1.580	.2550	.1180	.1370	13.48	1:0.18:7.7
	Sept. 8, 1923	Sept. 19	7.64	4.94	12.58	.734	.1318	.0511	.0807	15.99	1:0.18:17.1
Red June	July 15, 1922	July 17	6.01	1.84	7.85	.551	.1584	.0603	.0981	10.56	1:0.28:14.2
	July 20, 1923	July 23	6.06	2.93	8.99	.686	.1373	.0713	.0660	10.30	1:0.2:13.6
	July 28, 1924	Aug. 13	6.54	2.43	8.97	.350	.1160	.0390	.0790		1:0.33:25.7
Red Siberian	Aug. 1, 1922	Aug. 7	9.24	4.06	13.30	1.351	.5283	.2340	.2943	16.36	1:0.39:9.8
	Sept. 3, 1923	Sept. 18	8.18	4.80	12.98	.938	.6600	.4320	.2280	16.22	1:0.7:13.8
	Sept. 10, 1924	Sept. 16	7.49	2.13	9.62	.605	.5450	.3985	.1465		1:0.9:15.9
Red Sweet	Sept. 3, 1923	Sept. 11	8.66	3.98	12.64	.175	.1740	.0800	.0940	14.10	1:0.99:72.2
Ruby Gem	July 25, 1922	July 26	6.44	1.85	8.29	.769	.1635	.0399	.1236	10.72	1:0.21:10.7
	Aug. 1, 1923	Sept. 12	6.00	4.94	10.94	.676	.1680	.0643	.1037	12.73	1:0.24:16.2
	Aug. 11, 1924	Aug. 25	6.66	1.84	8.50	.400	.1172	.0584	.0588		1:0.29:21.2
Soulard Crab	Oct. 16, 1922	Oct. 18	5.90	1.39	7.29	.761	.1980	.1305	.0675	11.00	1:0.26:9.5
	Oct. 4, 1924	Dec. 6	5.28	1.71	6.99	.616	.2860	.1835	.1025	10.16	1:0.46:11.3
	Nov. 3, 1925	Nov. 25	6.18	2.36	8.54	.633	.3420	.1965	.1455	11.15	1:0.54:13.5
Springdale	Oct. 20, 1922	Oct. 24	7.45	2.73	10.18	.193	.1274	.0557	.0717	13.84	1:0.66:52.7
	Oct. 20, 1923	Oct. 31	8.44	4.28	12.72	.558	.1535	.0395	.1140	13.98	1:0.27:22.8
	Nov. 4, 1924	Dec. 2	7.70	1.66	9.36	.325	.1290	.0487	.0803		1:0.4:28.8
Stump	Aug. 23, 1923	Aug. 30	6.36	5.12	11.48	.534	.1300	.0625	.0675	12.63	1:0.24:21.5
Summer Pippin	July 25, 1923	...do....	7.36	2.43	9.79	.792	.1500	.0765	.0735	11.39	1:0.19:12.3
Tetofski	June 25, 1922	June 30	5.10	2.38	7.48	.614	.1451	.0511	.0940	8.68	1:0.23:12.1
Titus Pippin	Sept. 23, 1922	Nov. 13	7.52	2.80	10.32	.279	.1717	.0933	.0784	13.98	1:0.61:37.0
	Sept. 28, 1923	Oct. 2	7.32	2.87	10.19	.582	.1182	.557	.0625	13.14	1:0.2:17.5
	Oct. 1, 1925	Oct. 26	9.32	1.92	11.24	.314	.1390	.0630	.0760	14.74	1:0.44:35.7

Traders	July 28, 1922	July 31	6.87	2.01	8.88	.717	.1512	.0225	.1287	11.16	1:0.21:12.3
	Aug. 16, 1923	Aug. 24	8.34	4.92	13.26	.529	.2030	.1345	.0685	15.07	1:0.39:25.5
	Aug. 29, 1924	Sept. 4	6.51	2.61	9.12	.733	.1005	.0293	.0712		1:0.13:12.4
Transcendent	Aug. 1, 1922	Aug. 7	8.32	1.66	9.98	1.140	.3617	.1696	.1921	11.84	1:0.317:8.7
	Aug. 20, 1923	Aug. 27	7.44	4.27	11.71	.783	.2910	.1662	.1248	14.44	1:0.37:14.9
	Aug. 18, 1924	Aug. 21	7.64	1.16	8.80	.417	.3180	.1230	.1950		1:0.76:21.1
	Aug. 6, 1925	Aug. 10	6.41	3.68	10.09	.776	.2136	.1036	.1100		1:0.27:13
Trenton Early	July 10, 1922	July 17	6.68	1.74	8.42	.984	.1400	.0583	.0817	10.63	1:0.14:8.5
	Aug. 10, 1923	Aug. 13	6.02	1.08	7.10	1.027	.1725	.0623	.1102		1:0.168:6.9
	Aug. 3, 1922	Aug. 7			10.19	.199	.1727	.0593	.1134	11.79	1:0.86:51.2
Trumbull	Aug. 29, 1923	Sept. 5	9.18	3.75	12.93	.221	.1700	.0935	.0765	14.34	1:0.76:58.5
	Aug. 29, 1924	Sept. 12	6.86	3.02	9.88	.133	.1251	.0564	.0687	11.34	1:0.94:74.2
Whitney	July 26, 1922	July 26	7.04	3.16	10.20	.678	.1400	.0276	.1124	13.04	1:0.2:15
	Aug. 2, 1923	Oct. 25	10.40	4.42	14.82	.542	.1055	.0515	.0540	16.62	1:0.19:27.3
Williams	July 25, 1923	July 30	6.67	4.10	10.77	.551	.1420	.0695	.0725	12.32	1:0.25:19.5
	July 29, 1924	Aug. 6	6.67	2.15	8.82	.296	.1275	.0606	.0669		1:0.43:29.8
	July 21, 1925	July 25	6.71	1.53	8.24	.387	.0856	.0311	.0545		1:0.22:21.3
Wild Red	Nov. 1, 1923	Nov. 8	6.67	3.49	10.16	.727	.3170	.1869	.1301	11.36	1:0.43:13.9
	Oct. 18, 1924	Nov. 25	5.86	6.66	12.52	.723	.2765	.1425	.1340		1:0.38:17.3
	Oct. 16, 1922	Oct. 18	8.76	1.20	9.96	.114	.1447	.0860	.0587	12.04	1:1.27:87.3
Winter Paradise	Oct. 19, 1923	Oct. 25	8.84	3.34	12.18	.132	.1435	.0759	.0676	14.15	1:1.08:92.2
	Oct. 28, 1924	Nov. 1	7.02	2.40	9.42	.107	.1667	.0595	.1072		1:1.56:88.0
	Oct. 10, 1925	Oct. 31	9.32	2.16	11.48	.092	.1620	.0670	.0950	13.84	1:1.76:124.7
Worcester Pearmain	Aug. 14, 1923	Aug. 17	12.44	1.25	13.69	.328	.2930	.1470	.1460	14.85	1:0.89:41.1
	Aug. 1, 1922	Aug. 7	10.88	1.66	12.54	1.154	.4374	.1952	.2422	15.40	1:0.38:10.9
Yellow Siberian	Sept. 6, 1923	Sept. 18	6.67	4.53	11.20	1.010	.3450	.1982	.1468	13.02	1:0.34:11.0
	Aug. 29, 1924	Sept. 19	7.82	3.20	11.02	.593	.3760	.2335	.1425		1:0.63:18.5
	June 26, 1922	June 30	6.34	1.56	7.90	.680	.1942	.1125	.0817	9.24	1:0.28:11.6
Yellow Transparent	July 9, 1923	July 18	7.35	2.07	9.42	1.400	.2270	.0876	.1394	12.17	1:0.16:6.7
	July 23, 1924	July 30	5.31	.77	6.08	.439	.1360	.0380	.0980	9.69	1:0.31:13.8
	July 11, 1925	July 17	5.92	1.61	7.53	.486	.0753	.0255	.0498		1:0.15:15.5

ASTRINGENT FRUITS OF LOW ACID CONTENT

A relatively small group of varieties are comparable with the French cider-apple varieties previously discussed in that they are fairly high in sugar content while low in acidity, 0.5 per cent or less. In total astringency they as a class fall somewhat below most of the French cider group. Some of them have amounts exceeding those found in such French apples as Fréquin Lajoie, Fréquin Rouge, Précoce de Tunis, Rossignol, and Rouge du Landel, while others only slightly exceed 125 mgm. per 100 c. c. Examples of this class are Algérienne, Golden Sweet, Hog Island, Kentucky Sweet, Muster, Nain de Mahon, New England Pigeon, Ramsdell Sweet, Red Sweet, Trumbull, Winter Paradise, and Worcester Pearmain. By reason of their generally low acidity, they may be employed in combination with other apples of ordinary cultivated types for the same purpose as the French group, namely, the addition of astringency without material modification of sugar content, or for the addition of astringency together with the reduction of acid content. The fact that a few of them—for example, Golden Sweet and Ramsdell Sweet—are old varieties which have fairly wide distribution in home orchards and which are carried by nurserymen, may make them more easily obtainable than are the French apples of like acid-astringency-sugar ratios.

ASTRINGENT FRUITS OF MEDIUM ACID CONTENT

A second group of the apples for which analyses are presented in Table 4 is made up of fruits whose juices have an acid content ranging from 0.5 to 0.75 per cent. This group includes Augustine, Arctic, Early Strawberry, Florence, Hyslop, Lyman, Soulard Crab, Whitney, Wild Red, and a number of others. It will be noted that a considerable number of the older and more widely known and cultivated crab apples are included in this list. The range of acidity in the group is somewhat above the general average of the juices of commercial varieties. Their function will be that of increasing both acidity and astringency of juices to which they are added. Some of the group, as Florence, Hyslop, Lyman, and Soulard Crab, have astringent contents comparable with those of the first group or with the French cider fruits, but most of the group are considerably below these in tannin content.

HIGHLY ACID ASTRINGENT APPLES

A third group consists of apples which combine an acidity of 0.8 per cent or more with a considerable degree of astringency. Canada Reinette, Entz, Hogg, Jumbo, Martha, and Red Astrachan are typical of this group. Red Siberian and Yellow Siberian, while falling below the limit of 0.8 per cent acidity in one year (1924) are in normal years to be ranked with this group. In astringent content they present a rather wide range, Red Siberian, Yellow Siberian, and Entz having amounts comparable with the French apples, while Canada Reinette only slightly exceeds 125 mgm. per 100 c. c. In consequence the group contains material which can be employed to produce any desired degree of astringency and acidity in bulk material of subacid or sweet character. As several of the varieties of the group are old widely distributed apples which are grown both in home

orchards and in a limited commercial way, some one or another should prove to be adapted to cultivation in almost any of the important apple-producing districts.

Analytical data upon the juices of the wild Southern Crab, *Pyrus angustifolia*, have been included in the table for the purpose of calling attention to the very unusual character of the fruit of this species. Repeated analyses of the fruit of two trees on the Arlington farm grounds, continued over a series of years and checked by analyses on the fruit of the parent tree, are consistent in showing a remarkably low sugar content, an amount of astringent substances equaling or exceeding that of any of the French apples, and a titratable acidity approximating 2.5 per cent. The astringent material is somewhat suggestive of that of the French bitter-sweet group, having the bitterness characteristic of Amère du Surville and Julian de Paulmier. In amount of titratable acidity it very greatly exceeds any other apple thus far analyzed in this laboratory, having approximately twice the amount found in the most highly acid named varieties such as Entz, Hogg, Red Siberian, and Yellow Siberian. The fruit is of course quite inedible, but is occasionally gathered and used by housewives, small amounts being added to cultivated apples in making preserves or apple butter. The fruit keeps almost indefinitely in common storage and can be found under the fallen leaves late in March or April in perfectly sound condition and with acidity and astringency well preserved. Experiments with this apple have shown that its juice, added in very small quantities to such insipidly sweet juices as those of Delicious and other varieties lacking in both acid and tannin, greatly improves their palatability. In mixtures in which the quantity added does not exceed 3 to 5 per cent the bitterness which is so pronounced in the undiluted crab juice is subdued and becomes very agreeable, thus suggesting the effect of the bitter-sweet French apple juices.

SUMMARY

Attention has been called to the difficulties encountered in the manufacture of certain products from apples as a consequence of the prevailingly subacid and nonastringent character of the commercially popular varieties and of most of those widely grown in home orchards.

The necessity for giving more attention to varieties of highly acid and astringent character for blending with cultivated sorts in order to produce properly balanced products has been pointed out. As a contribution toward the information necessary as a guide in such work, analytical data have been presented upon 21 French cider-apple varieties grown at Blacksburg, Va., and at the Arlington Experiment Farm, Rosslyn, Va., and upon 61 astringent apple varieties of various origins grown at Arlington. That the various French cider apples undergo no very large or constant modification in their chemical character when grown either in the Virginia highlands at an elevation of 2,170 feet or a few feet above sea level at the Arlington farm is clear from comparisons of the analyses with the data from analyses made by French workers.

The 61 varieties of American, Siberian, and other apples which have been subjected to study may be grouped into three classes on

the basis of their titratable acidity as fruits of low, medium, and high acidity. These three groups will have somewhat different possibilities of usefulness. Each group contains varieties presenting a fairly wide range in content of astringent substances, as indicated by the range in acid-astringency-sugar ratios in each group. The varieties studied include most of the widely cultivated crab apples, together with others less widely known or confined to varietal collections. Taken collectively, the material discussed presents such a range of chemical character that it is possible to make selections adapted to meet almost any conceivable requirement of the manufacturer of apple products.

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CHEMICAL COMPOSITION OF THE JUICES OF SOME AMERICAN APPLES[1]

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INTRODUCTION

The literature of American pomology is notably lacking in data upon the chemical composition of much of the material with which it deals. Such information as is available is largely derived from studies of a relatively small number of varieties of outstanding commercial importance. The focusing of attention upon these in connection with studies of development, ripening, and behavior in storage has resulted in the accumulation of fairly complete data in regard to their chemical composition. At the same time the study of varieties of lesser commercial importance has been neglected. Only a very small percentage of the 2,500 to 3,000 named varieties of apples recognized by authorities have ever been subjected to chemical analysis. The standard textbooks usually contain lists of apples recommended for home orchards or for commercial planting in specified districts, but for many of these no results of chemical analysis have been recorded. In consequence, the varietal descriptions found in the manuals, while giving such details of physical appearance and character of the various fruits as permit recognition by the student, can go no further in their attempts at chemical characterization than the use of the rather vague terms "subacid," "tart," "mildly acid," "sweet," and the like. Such descriptions, however adequate they may be for the purposes for which they are designed, are of little use when the fruit is considered as raw material to be converted into various preserved food products. The uses which may advantageously be made of a given variety for manufacturing purposes depend in some degree upon its physical character, but in much larger degree upon its chemical composition. In the absence of detailed chemical data upon more than a small number of varieties of apples, the manufacture of food products is rather rigidly confined to these known varieties. The necessity for the production of uniform, standardized products forbids random employment of unknown material, and in consequence a very large volume of potentially valuable raw material will remain unused until investigation of its chemical character yields data indicating its possibilities.

REVIEW OF LITERATURE

Most of the analyses of apple juices made by American workers reported in the literature up to 1904 have been assembled by Van Slyke (*12*).[2] He reprinted analyses made by Browne (*5*) at the

[1] Received for publication July 28, 1926; issued May, 1928. This paper is the sixth in a series of studies on fruit juices.

[2] Reference is made by number (italic) to "Literature cited," p. 417.

Pennsylvania Agricultural Experiment Station, by Burd at Washington, D. C., for Alwood and his collaborators (*1*, *3*), and by Davidson at the Virginia Agricultural Experiment Station (*2*), together with a number of analyses made at the New York Agricultural Experiment Station by Le Clerc and Van Slyke (*12*). His tables include data on 75 dessert or culinary apples and 10 crabs, and his paper would appear to contain the most extensive collection of analyses of American apples, either of whole fruit or of juices, to be found in the literature. Richards (*11*) published analyses of the whole fruit of about 18 varieties in 1887, and Browne (*5*) has made similar analyses of 25 varieties. Bigelow, Gore, and Howard (*4*) made a large number of analyses on whole fruits of a limited number of varieties at various stages of maturity, and Gore (*9*) published analyses of the fresh juices of 11 varieties. Jones and Colver (*10*) made very extensive analytical studies of the composition of whole fruits of about 30 varieties of apples, grown under both irrigated and unirrigated conditions, and in these studies they included all the more important commercial varieties grown in Idaho.

In the papers cited the analyses usually included such varieties as were rather extensively cultivated in the State or locality in which the work was done, and a number of varieties are common to most or all of the lists. The more recent literature contains a considerable amount of analytical data in regard to a relatively small number of varieties already included in the older papers, so that altogether not more than 200 varieties are represented in the literature by analyses either of the whole fruit or of the juices.

PURPOSE OF THE PRESENT WORK

One phase of the investigations having to do with the utilization of surplus and unmarketable horticultural products in progress in the fruit and vegetable utilization laboratory of the Office of Horticulture has had as its object the accumulation of data upon the chemical composition of apples with especial reference to the availability of the various varieties and varietal groups as material for the making of unfermented juices for beverage purposes. It was felt that such a chemical survey of a large mass of unknown material would have very considerable scientific interest and would at the same time be of practical value by calling attention to the availability for manufacturing purposes of a large volume of material not being used.

The plan pursued in the accumulation of such data was primarily one of annual analysis of the expressed juices of a large number of varieties growing under controlled conditions, for the purpose of procuring data as to composition, as to the range and character of the variations in composition occurring from year to year, and as to the relation of such variations to the annual fluctuations in seasonal climatic conditions. The results of such a study of more than 200 varieties of apples, continued over a period of six years, and of 60 varieties of grapes over a period of five years, have been published (*6*, *7*).

With the twofold object of obtaining basic data upon the chemical composition of as large a number of apple varieties as possible and of discovering varieties of special characteristics which might make them valuable for special purposes, a large number of varieties have been

analyzed in this laboratory at various times since 1919. In a number of cases it was the original intention to make analyses annually in connection with the study to which reference has already been made, but the habit of irregular bearing, destruction of the crop by frosts, accidental injury or death of one of the trees, or limitation of time available for the work prevented this. In other cases no attempt was made to obtain analyses each year, but two analyses in different years were made when time permitted. Analyses of about 80 varieties of astringent apples are published elsewhere (*8*).

SOURCE OF MATERIAL

The fruit employed was grown in the apple variety collection of the Office of Horticulture, at the Arlington Experiment Farm, Rosslyn, Va., near Washington, D. C., except as otherwise noted, and consisted of sound tree-run samples of 1 to 5 bushels in quantity, of the mixed crop of the two trees of the variety. The samples were handled in all the details of storage, expression of juice, and analysis in the same manner as the material employed in the study already mentioned (*7*), which presents details as to the location of the orchard, character of soil, cultural treatment of the orchard, and methods of analysis employed. In so far as the joint judgment of two or three experienced persons could determine, all samples of fruit were pressed at a like stage of maturity, about halfway between picking-ripe condition and eating ripeness. In no case was an occasional crop subjected to analysis, that is, any pair of trees had in every case borne a crop approximating normal in amount in the year preceding that in which the crop was sampled for analysis.

SEASONAL CONDITIONS AS RELATED TO CHEMICAL COMPOSITION

The significance of the results of an isolated analysis of a variety of apple must remain questionable until much more is known as to the effect of latitude, climatic and soil conditions, culture, and fertilization upon the chemical composition of the fruit. In the absence of large numbers of analyses made at an identical stage of maturity upon a considerable number of varieties throughout the whole extent of their range and consequently under widely varying but measured conditions with respect to all the factors named, it can not be known to what extent an analysis of a variety grown at a given place and under a known set of conditions is indicative of what may be found under another set of conditions either at the same place or elsewhere.

Some background of information in regard to the extent to which variations in climatic conditions affect the composition of successive crops of the same trees is available for the analyses here presented. The six years 1920 to 1925, during which they were being accumulated, present several conditions approaching or equaling the maximum departures from normality recorded by the Washington station of the United States Weather Bureau during the 50-year period. While the year 1920 very closely approximated the normal in the amount and distribution of its rainfall, sunshine, and temperature, 1921 was the hottest year since 1891; 1923 had the largest number of hours of sunshine between March 1 and July 1 on record; 1924 had excessive precipitation with exceptionally low temperatures during the growing

season; and 1925 had a very large deficiency in spring and summer rainfall.

It has been shown elsewhere (7) that a large group of varieties upon which analyses were made during these years showed clear-cut mass responses to these differences in seasonal conditions. Employing the composition of the 1920 crop as a basis of comparison, the response consisted in an increase in sugar and acid content of the crop, accompanied by a decrease in astringent content, as the sunshine and degrees of temperature received during the growing season increased over the amounts received in 1920, and a decrease in sugar and acid and an increase in astringency as sunshine and temperature fell below those of 1920. In consequence of the control exerted over the chemical composition of the crop by the seasonal conditions, the crop of 1923 showed materially higher sugar content than did that of any other year, 1921 standing second, 1925 third, 1922 fourth, 1920 fifth, and 1924 sixth and lowest. High sugar content was accompanied by high acidity and low astringent content, low sugar content by low acidity and high astringent content, the comparative ranking of the crops of the various years with respect to each of these constituents being shown in Table 1.

TABLE 1.—*Comparative rank in total sugar, titratable acidity, and relative astringency of apples analyzed in 1920–1925*

Year	Total sugar [a]	Titratable acidity [a]	Relative astringency [b]	Year	Total sugar [a]	Titratable acidity [a]	Relative astringency [b]
1920	5	4		1923	1	2	4
1921	2	1		1924	6	5	1
1922	4	3	2	1925	3	6	3

[a] Ranking of 1 indicates maximum; 6, minimum.
[b] Data upon relative astringency were not obtained in 1920 and 1921; hence the ranking in these items is from 1, the maximum, to 4, the minimum.

Since the material employed in the present analyses was grown in the same orchard in the same years and under the same conditions as that employed in the study from which the conclusions just stated were derived, it is a logical assumption that the fruit of the varieties here employed was affected in the same manner by the seasonal conditions. Consequently, analyses made in 1923 or 1921 will show a content of sugar which is high for the variety as grown in this orchard and which will be attained only under conditions exceptionally favorable for photosynthetic activity. Those made in 1924 or 1920 will show a minimum sugar content, reached only in years which are low in sunshine and temperature, while those made in 1922 or 1925 may be expected to present results near the middle of the range in composition of the variety as grown in this locality. The correlation of sugar content with acidity and astringency content, already mentioned, should also be found to obtain in these results.

The data of Table 2 contain material for testing the validity of these assumptions, since a considerable number of the varieties were analyzed in two or in three years of the period, in some cases in consecutive years, in others with an interval of one or two years between. Comparison of the results for the individual varieties, or of a group of varieties analyzed in the same years, will at once indicate whether the individual or the group behaved in accordance with the assumptions just made.

TABLE 2.—*Analytical data for 98 varieties of apples grown at the Arlington Experiment Farm, Rosslyn, Va., 1920–1924*

Variety	Date of picking	Date analyzed	Constituents (per cent)								Acid-astringency-sugar ratio
			Reducing sugar	Sucrose	Total sugar	Acid as malic	Total astringency	Tannin	Non-tannins	Total solids	
Augustine	Sept. 3, 1923	Sept. 18	10.42	3.82	14.24	0.725	0.2182	0.0999	0.1183	16.09	1:0.3:19.6
Banks Gravenstein	July 2, 1922	July 7	6.90	2.14	9.04	.875	.1144	.0071	.1073	10.88	1:0.13:10.3
	Aug. 4, 1923	Aug. 13	7.78	2.40	10.18	.715	.1117	.0518	.0599	11.82	1:0.15:14.2
Barbarie [a]	Sept. 7, 1922	Oct. 21	10.10	1.54	11.64	.215	.3132	.1699	.1433	13.69	1:1.378:54.1
Bedan des Parts [a]	Aug. 28, 1923	Sept. 7	9.14	1.37	10.51	.146	.4070	.2745	.1325	11.40	1:2.75:54.1
Berry	Sept. 23, 1922	Nov. 14	9.64	5.32	14.96	.314	.1168	.0478	.0690		1:0.372:47.6
	Sept. 16, 1923	Sept. 28	9.14	6.22	15.36	.332	.0990	.0340	.0650	15.97	1:0.298:46.2
Bigg	Oct. 8, 1923	Oct. 23	6.96	3.88	10.84	.292	.0815	.0277	.0538	11.12	1:0.27:37.1
	Oct. 4, 1924	Oct. 14	6.11	3.63	9.74	.516	.0825	.0309	.0516		1:0.16:18.9
Bloomfield	Aug. 23, 1922	Aug. 29	7.09	1.40	8.49	.703	.1104	.0371	.0733	12.27	1:0.157:12.1
	Aug. 23, 1923	Sept. 5	7.06	3.02	10.08	.732	.1070	.0552	.0518	11.98	1:0.146:13.7
Blue Mountain	Aug. 14, 1922	Aug. 21	8.28	2.40	10.68	.812	.0572	.0041	.0531	11.95	1:0.07:13.1
	Sept. 11 1923	Sept. 22	6.08	3.25	9.33	.487	.0930	.0386	.0544	11.68	1:0.19:19.1
	Sept. 3, 1924	...do	6.09	2.65	8.74	.422	.0525	.0217	.0308		1:0.12:20.7
Bonne de Freuilles	Sept. 2, 1923	Sept. 19	7.73	3.61	11.34	.330	.3280	.2050	.1230	13.83	1:0.99:34.3
Bramtot	Sept. 11, 1922	Sept. 24	11.94	3.69	15.63	.316	.3385	.1735	.1650		1:1.07:49.4
	Sept. 6, 1923	Sept. 19	11.84	4.10	15.94	.187	.4600	.2432	.2168	17.82	1:2.46:85.2
Carlough	Oct. 26, 1922	Oct. 30	7.68	3.17	10.85	.285	.0956	.0363	.0593	13.01	1:0.37:42.3
	Nov. 14, 1924	Nov. 15	8.07	1.75	9.82	.223	.0498	.0194	.0304		1:0.22:44
Clawis	Oct. 6, 1921	Nov. 3	7.04	3.97	11.01	.577	.0735	.0150	.0585		1:0.127:19.1
Codorus	Oct. 4, 1923	Nov. 17	7.56	4.12	11.68	.452	.0822	.0326	.0496	13.60	1:0.181:25.8
	Aug. 13, 1920	Aug. 25	8.04	3.00	11.04	.424	.0861	.0366	.0495	12.95	1:0.2:26
Connet	Sept. 2, 1922	Sept. 13	8.25	5.01	13.26	.136	.1750	.0852	.0898	15.75	1:1.28:97.5
	Sept. 5, 1923	Sept. 16	6.76	5.56	12.32	.125	.1020	.0388	.0632		1:0.816:98.5
Cornell	Aug. 3, 1922	Aug. 4	7.86	2.22	10.08	.660	.1134	.0163	.0971	11.78	1:0.17:15.7
	Aug. 29, 1924	Sept. 8	6.96	2.05	9.01	.348	.1017	.0385	.0632		1:0.29:25.8
Cox Golden	Sept. 11, 1921	Sept. 29	6.90	3.02	9.92	.496	.0811	.0178	.0633		1:0.163:20
Cox No. 14	Sept. 18, 1922	Nov. 13	7.94	2.68	10.62	.289	.1115	.0522	.0593	12.79	1:0.38:36.7
Crookstem	Oct. 6, 1923	Oct. 19	7.08	3.08	10.16	.756	.0615	.0208	.0407	12.45	1:0.08:13.4
	Oct. 21, 1923	Oct. 31	7.82	3.51	11.33	.715	.0702	.0205	.0497	13.40	1:0.09:15.8
	Oct. 18, 1924	Nov. 20	8.55	3.21	11.76	.840	.0862	.0255	.0607		1:0.1:14
D'Avrolles [a]	Oct. 4, 1922	Oct. 18	11.53	1.83	13.36	.210	.4148	.2991	.1157	14.68	1:0.197:63.6
De Chataignier	Oct. 7, 1922	Oct. 20	8.54	2.02	10.56	.261	.1221	.0558	.0663	13.38	1:0.47:40.4
	Nov. 4, 1924	Nov. 27	8.36	2.14	10.50	.213	.1125	.0517	.0608		1:0.52:49.3
D'Eve	Sept. 14, 1923	Nov. 12	8.50	4.06	12.56	.770	.1090	.0618	.0472	15.92	1:0.142:16.3
Drop de 'Or	Aug. 10, 1922	Aug. 16	8.54	2.46	11.00	.525	.0960	.0204	.0756	11.98	1:0.18:20.9
Dulaney	Oct. 14, 1921	Oct. 30	6.63	4.73	11.36	.531	.0980	.0223	.0757	13.83	1:0.184:21.4
Dumelow	Aug. 25, 1922	Aug. 29	8.33	1.15	9.48	1.116	.1726	.0587	.1139	12.93	1:0.154:8.5
	Sept. 16, 1923	Sept. 28	9.86	2.59	12.45	.957	.1160	.0510	.0650	14.55	1:0.121:13.0
Early Edward	July 20, 1922	July 25	7.64	.56	8.20	.581	.1573	.0643	.0930	11.70	1:0.27:14.1
	July 30, 1923	July 30	6.78	2.58	9.36	.821	.1200	.0585	.0615	11.41	1:0.146:11.4

[a] Fruit used grown at Virginia Agricultural Experiment Station, Blacksburg, Va.

TABLE 2.—*Analytical data for 98 varieties of apples grown at the Arlington Experiment Farm, Rosslyn, Va., 1920–1924*—Continued

Variety	Date of picking	Date analyzed	Constituents (per cent)								Acid-astringency-sugar ratio
			Reducing sugar	Sucrose	Total sugar	Acid as malic	Total astringency	Tannin	Non-tannins	Total solids	
Elstun	Oct. 8, 1923	Oct. 10	7.72	4.24	11.96	0.337	0.0815	0.0200	.0615	14.06	1:0.24:35.4
	Oct. 4, 1924	Oct. 16	6.91	3.89	10.80	.212	.0884	.0352	.0532		1:0.41:50.9
Ensee	Aug. 17, 1921	Sept. 6	7.80	2.80	10.60	1.070	.0546	.0040	.0506	12.44	1:0.05:9.9
	Sept. 16, 1923	Sept. 28	8.40	5.17	13.57	.715	.0947	.0342	.0605	14.94	1:0.132:18.9
Excelsior	Aug. 9, 1920	Aug. 18	7.70	1.51	9.21	.685	.0914	.0180	.0734		1:0.133:13.4
Fall Cheese	Oct. 9, 1923	Oct. 17	8.04	4.34	12.38	.417	.0831	.0300	.0531	13.21	1:0.2:29.6
Fall Swaar	Aug. 3, 1922	Aug. 4	7.35	3.34	10.69	.816	.1287	.0592	.0695	11.94	1:0.16:13.1
	Aug. 20, 1923	Aug. 27	6.29	4.45	10.74	.478	.1055	.0555	.0500	11.88	1:0.22:22.4
Ferdinand	Oct. 12, 1922	Dec. 6	9.59	2.63	12.22	.378	.0773	.0303	.0470	15.17	1:0.2:29.6
	Sept. 23, 1924	Nov. 22	10.06	2.46	12.52	.291	.1072	.0480	.0592		1:0.368:43
Foote	Oct. 8, 1923	Oct. 17	7.18	3.89	11.07	.630	.1013	.0293	.0720	13.34	1:0.16:17.6
	Oct. 8, 1924	Oct. 10	7.73	3.29	11.02	.852	.1140	.0360	.0780		1:0.133:12.9
Fréquin Lajoie [a]	Aug. 28, 1923	Sept. 10	7.49	2.89	10.38	.318	.2780	.1515	.1265	11.56	1:0.87:32.3
Fréquin Rouge	Sept. 16, 1923	Sept. 29	9.71	4.05	13.76	.370	.1568	.0568	.1000	15.58	1:0.42:37.2
Fulcher	Oct. 10, 1922	Oct. 11	8.29	4.04	12.33	.697	.1113	.0368	.0745	12.95	1:0.16:17.6
	Sept. 7, 1924	Sept. 20	7.44	3.90	11.34	.552	.1170	.0455	.0715		1:0.21:20.5
Gascoyne	Sept. 16, 1923	Nov. 22	7.54	5.34	12.88	.458	.1050	.0545	.0505	14.88	1:0.229:28.1
Gem Sweet	Aug. 14, 1923	Aug. 17	3.64	3.75	7.39	.128	.1000	.0413	.0587	8.24	1:0.78:57.7
Gladstone	July 20, 1922	July 25	6.48	1.68	8.16	.869	.1829	.0562	.1267	11.36	1:0.21:9.4
	Aug. 5, 1924	Aug. 8	7.22	1.12	8.34	.733	.1930	.0857	.1073		1:0.263:11.3
Golden Noble	Aug. 12, 1922	Aug. 21	8.08	3.15	11.23	1.131	.0674	.0194	.0480	13.49	1:0.59:9.9
	Aug. 13, 1923	Aug. 30	5.06	4.52	9.58	.735	.0720	.0342	.0378	10.91	1:0.98:13.0
Grand Sultan	July 3, 1922	July 11	6.61	2.40	9.01	.979	.1327	.0565	.0762		1:0.14:9.2
	July 6, 1923	July 13	5.86	1.05	6.91	.894	.1321	.0520	.0801		1:0.14:7.7
Hackman	Aug. 19, 1922	Aug. 25	7.15	2.53	9.68	.611	.0950	.0122	.0828	12.80	1:0.154:15.8
	Oct. 8, 1923	Oct. 12	7.43	3.63	11.06	.392	.0850	.0250	.0600	12.59	1:0.217:28.2
Harwell	Sept. 3, 1921	Oct. 4	7.03	3.86	10.89	.478	.0717	.0142	.0575		1:0.15:22.8
	Oct. 2, 1924	Oct. 18	7.68	2.62	10.30	.364	.1010	.0436	.0574	12.15	1:0.27:28.3
Haskell	Aug. 24, 1920	Sept. 11	7.14	1.80	8.94	.160	.1018	.0293	.0725		1:0.63:55.8
	Sept. 15, 1924	Sept. 24	6.37	1.95	8.32	.094	.1305	.0755	.0550		1:1.38:88.5
Hewes	Sept. 3, 1923	Sept. 18	4.98	2.32	7.30	1.050	.1695	.1045	.0650	8.33	1:0.161:6.95
Hog Island	Aug. 3, 1922	Aug. 4	8.91	1.19	10.10	.212	.1921	.0460	.1461	12.76	1:0.906:47.6
	Aug. 27, 1923	Sept. 4	8.20	3.37	11.57	.177	.1261	.0533	.0728	12.48	1:0.712:65.3
Hope	Aug. 7, 1922	Aug. 8	7.84	2.16	10.00	.792	.0991	.0102	.0889	11.91	1:0.125:12.6
Isham	Aug. 23, 1922	Aug. 29	6.22	2.98	9.20	.393	.1096	.0337	.0759	11.88	1:0.27:23.4
	Sept. 8, 1923	Sept. 19	7.18	4.93	12.11	.316	.1010	.0378	.0632	13.99	1:0.32:38.3
Jacob Sweet	Sept. 28, 1922	Dec. 22	8.22	2.42	10.64	.219	.0427	.0205	.0222	12.47	1:0.194:48.5
Jenks	Aug. 19, 1923	Nov. 5	8.05	2.11	10.16	.330	.1070	.0455	.0615		1:0.32:30.8
	Aug. 22, 1924	Aug. 27	7.49	2.23	9.72	.272	.0456	.0321	.0135		1:0.16:35.7
Jerusalem Sweet	Aug. 16, 1922	Aug. 17	8.71	2.05	10.76	.370	.1256	.0050	.1206	12.50	1:0.33:29
	Aug. 27, 1923	Sept. 24	8.20	3.26	11.46	.258	.1220	.0315	.0905	12.79	1:0.473:44.4
John Sharp	Nov. 4, 1923	Nov. 5	8.20	4.17	12.37	.745	.0952	.0282	.0670	13.78	1:0.128:16.6
	Oct. 4, 1924	Oct. 15	7.01	2.50	9.51	.805	.1115	.0403	.0712		1:0.138:11.8

Jones's Green	Aug. 23, 1923	Sept. 12	8.94	1.24	10.18	.404	.1290	.0640	.0650	11.17	1:0.32:25.2
	Sept. 3, 1924	Oct. 6	7.80	1.66	9.46	.517	.0910	.0370	.0540		1:0.174:18.3
Jouveaux [a]	Sept. 2, 1923	Sept. 7	9.70	1.44	11.14	.178	.3480	.2215	.1265	14.10	1:1.95:62.5
July	July 20, 1923	July 26	4.87	1.97	6.84	.612	.1410	.0597	.0813	7.62	1:0.23:11.2
Jumbo	July 20, 1922	July 25	6.48	1.30	7.78	.852	.1645	.0777	.0868	11.84	1:0.193:47.2
	July 16, 1923	Aug. 3	7.26	2.66	9.92	.880	.1540	.0745	.0795	11.95	1:0.175:64.4
Kennedy	July 21, 1923	July 30	5.49	3.92	9.41	.568	.1368	.0659	.0709	10.80	1:0.24:16.5
Kentucky Long Stem	Oct. 16, 1923	Oct. 19	8.30	4.16	12.46	.450	.0986	.0241	.0745	14.36	1:0.22:27.6
	Oct. 18, 1924	Nov. 8	7.14	3.16	10.30	.447	.1130	.0582	.0548		1:0.25:23.0
Kittagekee	Sept. 27, 1923	Sept. 29	8.92	4.23	13.15	.587	.0870	.0283	.0587	14.22	1:0.15:22.4
	Sept. 15, 1924	Oct. 15	8.70	3.08	11.78	.570	.1057	.0345	.0712		1:0.18:20.6
Launette [a] [b]	Sept. 1, 1923	Sept. 7	8.55	3.08	11.63	.229	.5025	.2865	.2160		1:2.19:50.8
Do	Sept. 15, 1924	Sept. 24	9.43	1.21	10.64	.219	.4700	.2450	.2250	11.93	1:2.14:44
Launette Grosse	Sept. 11, 1923	Sept. 18	11.98	3.18	15.16	.299	.6865	.3085	.3780	17.88	1:2.29:50.7
Lowland Raspberry	July 16, 1923	July 23	8.08	3.62	11.70	1.010	.2100	.1340	.0760	12.21	1:0.21:11.5
	Aug. 5, 1924	Aug. 8	7.80	.61	8.41	.682	.2000	.0735	.1265		1:0.29:12.3
Loddington	Aug. 14, 1922	Aug. 16	6.85	2.85	9.70	1.048	.0971	.0174	.0797	10.64	1:0.092:9.2
	Aug. 13, 1923	Aug. 21	7.18	2.74	9.92	1.065	.1132	.0500	.0632	10.36	1:0.106:23
Loy	Sept. 27, 1923	Oct. 9	7.44	3.34	10.78	.727	.0694	.0084	.0610	13.43	1:0.095:14.8
	Sept. 28, 1924	Oct. 20	6.60	3.10	9.70	.520	.0625	.0095	.0530		1:0.12:18.6
Lubsk Reinette	July 13, 1920	July 19	7.55	1.47	9.02	.533	.1432	.0594	.0838		1:0.26:16.9
	July 10, 1923	...do...	6.02	2.74	8.76	.607	.1500	.0568	.0932		1:0.24:14.4
Lucinda	Sept. 16, 1923	Sept. 27	6.64	3.81	10.45	.670	.0720	.0097	.0623	11.94	1:0.107:15.6
	Oct. 8, 1924	Oct. 10	6.65	3.73	10.38	.482	.0970	.0430	.0540		1:0.2:21.5
Magnet	Sept. 8, 1923	Sept. 22	7.34	3.76	11.10	.343	.1272	.0694	.0578	12.74	1:0.37:32.3
	Sept. 13, 1924	Sept. 18	7.18	3.73	10.91	.294	.0955	.0422	.0533		1:0.32:37.1
Mason Orange	Oct. 8, 1923	Nov. 5	8.18	3.78	11.96	.425	.1065	.0551	.0514	13.92	1:0.25:28.1
	Oct. 4, 1924	Oct. 11	7.72	3.30	11.02	.427	.0774	.0206	.0568		1:0.18:25.8
McCord	Sept. 6, 1922	Sept. 20	6.64	3.01	9.65	.426	.0808	.0052	.0756	12.22	1:0.19:22.6
	Oct. 8, 1924	Oct. 11	7.67	1.83	9.50	.510	.1110	.0430	.0680		1:0.21:18.6
Melo Annurco	Oct. 12, 1923	Oct. 19	8.30	5.03	13.33	.466	.0925	.0241	.0684	16.33	1:0.2:28.6
	Oct. 28, 1924	Nov. 20	6.93	2.37	9.30	.307	.1080	.0507	.0573		1:0.35:30.3
Melo Lemoncelli	Oct. 9, 1923	Oct. 17	7.50	5.12	12.62	.612	.1055	.0365	.0688	15.88	1:0.17:20.6
Moulin-à-Vent [a] [b]	Sept. 1, 1923	Sept. 7	7.46	2.31	9.77	.163	.4050	.2330	.1720	11.41	1:2.48:60
Nain de Mahon	Sept. 13, 1923	Sept. 27	7.68	4.32	12.00	.360	.1322	.0373	.0949	14.36	1:0.367:33.3
	Sept. 15, 1924	Nov. 4	8.78	6.58	15.36	.172	.2050	.1020	.1030		1:1.19:89.3
New England Pigeon	Aug. 19, 1922	Aug. 25	7.12	2.38	9.50	.395	.1451	.0542	.0909	12.09	1:0.367:24.1
	Sept. 8, 1923	Sept. 23	7.46	1.53	8.99	.212	.1525	.0553	.0972	10.42	1:0.72:42.4
Nonesuch	Aug. 3, 1922	Aug. 4	6.87	2.59	9.46	1.573	.1420	.0368	.1052	11.62	1:0.09:6.0
	Aug. 21, 1924	Aug. 24	4.42	1.83	6.25	.650	.1205	.0619	.0586	8.12	1:0.185:9.6
Omont	Sept. 11, 1923	Sept. 19	12.17	3.45	15.62	.256	.6400	.3290	.3110	21.13	1:2.5:61
	Sept. 7, 1924	Sept. 18	8.50	1.87	10.37	.164	.2680	.1060	.1620		1:1.63:95
Palouse	Aug. 11, 1922	Aug. 14	8.02	2.60	10.62	.864	.0845	.0100	.0745	11.64	1:0.97:12.3
Park	Oct. 13, 1922	Oct. 17	8.18	3.44	11.62	.291	.0772	.0229	.0543	13.66	1:0.26:39.8
	Nov. 4, 1924	Nov. 13	7.64	2.60	10.24	.264	.0735	.0254	.0481		1:0.27:38.7
Parry White	Aug. 16, 1923	Aug. 21	6.90	2.14	9.04	.800	.1035	.0481	.0554	10.51	1:0.129:11.3
Passe Reine	July 13, 1922	July 28	7.86	1.61	9.47	.988	.2984	.1339	.1645	11.20	1:0.3:9.58
Petite Douce Rousse	Sept. 16, 1924	Sept. 24	8.20	3.32	11.52	.202	.4110	.2628	.1482	14.36	1:2.03:57
Plott (seedless)	Aug. 7, 1922	Aug. 8	7.86	.88	8.74	1.021	.1492	.0338	.1154	11.78	1:0.146:8.56
	Aug. 31, 1923	Sept. 4	6.29	4.05	10.34	.770	.0918	.0296	.0622	10.34	1:0.12:13.43

[a] Fruit used grown at Virginia Agricultural Experiment Station, Blacksburg, Va.

[b] Slightly past prime condition for pressing when received at the laboratory.

TABLE 2.—*Analytical data for 98 varieties of apples grown at the Arlington Experiment Farm, Rosslyn, Va., 1920–1924*—Continued

Variety	Date of picking	Date analyzed	Constituents (per cent)							Acid-astringency-sugar ratio
			Reducing sugar	Sucrose	Total sugar	Acid as malic	Total astringency	Tannin	Non-tannins	Total solids	
Rambour Papeleu	Sept. 19, 1923	Sept. 24	6.48	3.26	9.74	0.156	0.1255	0.0563	0.0692	11.06	1:0.8:62.4
	Sept. 13, 1924	Oct. 6	7.38	2.54	9.92	.253	.0927	.0217	.0710	----------	1:0.366:39.2
Randolph	July 18, 1923	July 23	6.64	3.50	10.14	.632	.1215	.0711	.0504	10.98	1:0.19:16
	Aug. 4, 1924	Aug. 7	7.40	2.27	9.67	.434	.1170	.0308	.0862	----------	1:2.7:22.3
Red Eiser	Sept. 8, 1923	Sept. 19	7.64	4.94	12.58	.734	.1318	.0511	.0807	15.99	1:0.18:17.1
Reine des Pommes [a]	Sept. 17, 1923	Sept. 22	8.15	3.18	11.33	.189	.4440	.2855	.1585	14.05	1:2.34:60.0
Do	Sept. 1, 1923	Sept. 7	8.80	3.10	11.90	.329	.5945	.3965	.1980	13.80	1:1.8:36.0
Rossignol [a]	Sept. 16, 1922	Oct. 21	10.23	1.99	12.22	.332	.1982	.0956	.1026	15.37	1:0.06:36.8
Rouge du Landel [a] [b]	Sept. 1, 1923	Sept. 8	8.20	1.80	10.00	.355	.2920	.1452	.1468	11.76	1:0.823:28.2
Do	Sept. 7, 1923	Sept. 20	8.23	1.45	9.68	.296	.2512	.1002	.1510	11.93	1:0.848:32.7
	Sept. 7, 1924	Sept. 15	7.58	2.91	10.49	.171	.2920	.1575	.1345	----------	1:1.7:61.3
Shannon	Oct. 3, 1924	Oct. 14	6.58	3.75	10.33	.446	.0675	.0245	.0430	----------	1:0.15:23.1
Smith Early Red	Sept. 13, 1924	Sept. 27	5.74	3.40	9.14	.575	.0755	.0250	.0505	11.22	1:0.13:15.9
Stump	Aug. 23, 1923	Aug. 30	6.36	5.12	11.48	.534	.1300	.0625	.0675	12.63	1:0.24:21.5
Summer Extra	July 26, 1922	July 27	7.76	1.53	9.29	.645	.1430	.0296	.1134	11.04	1:0.22:14.4
	Aug. 13, 1923	Aug. 17	8.28	3.29	11.57	.655	.1187	.0467	.0720	13.34	1:0.18:17.6
Summer Pippin	July 25, 1923	Aug. 30	7.36	2.43	9.79	.792	.1500	.0765	.0735	11.39	1:0.19:12.3
Sweet Fameuse	Sept. 16, 1923	Dec. 5	9.13	2.55	11.68	.164	.1200	.0713	.0487	14.68	1:0.73:71.2
	Sept. 15, 1924	Oct. 2	6.20	1.89	8.09	.225	.0996	.0498	.0498	----------	1:0.44:32.0
Taupaki	Aug. 29, 1922	Sept. 5	8.96	1.20	10.16	.680	.0869	.0343	.0526	12.66	1:0.13:14.9
	Sept. 8, 1923	Sept. 22	6.90	3.21	10.11	.685	.1132	.0457	.0675	11.96	1:0.16:14.7
Tidal	Oct. 12, 1923	Oct. 22	8.08	2.64	10.72	.290	.0806	.0334	.0472	11.13	1:0.27:36.9
	Oct. 18, 1924	Oct. 23	7.00	2.36	9.36	.288	.0648	.0202	.0446	10.47	1:.0225:32.5
Townsend	July 21, 1922	July 31	6.26	2.95	9.21	.740	.1134	.0235	.0899	11.61	1:0.15:12.4
	Aug. 3, 1923	Aug. 13	7.06	3.37	10.43	.462	.0855	.0342	.0513	11.66	1:0.18:22.5
Trenton Early	July 10, 1922	July 17	6.68	1.74	8.42	.984	.1400	.0583	.0817	10.63	1:0.142:8.5
	Aug. 10, 1923	Aug. 13	6.02	1.08	7.10	1.027	.1725	.0623	.1102	----------	1:0.168:6.9
Vanhoy	Oct. 26, 1923	Oct. 31	6.40	5.83	12.23	.607	.0924	.0324	.0600	12.72	1:0.15:20.1
	Oct. 28, 1924	Nov. 11	7.05	2.72	9.77	.498	.0905	.0415	.0490	----------	1:0.18:19.6
Whinery	Oct. 6, 1922	Oct. 30	8.06	5.00	13.06	.228	.0982	.0282	.0700	14.86	1:0.43:57.2
	Oct. 12, 1923	Oct. 26	7.64	4.36	12.00	.286	.0642	.0230	.0412	12.97	1:0.224:41.9
Whitney	July 26, 1922	July 26	7.04	3.16	10.20	.678	.1400	.0276	.1124	13.04	1:0.2:15
	Aug. 2, 1923	Oct. 25	10.40	4.42	14.82	.542	.1055	.0515	.0540	16.62	1:0.19:27.3
William Anderson	Sept. 15, 1923	Nov. 24	8.31	3.23	11.54	.580	.1092	.0511	.0581	14.04	1:0.188:19.9
	Sept. 22, 1924	Sept. 27	5.86	2.62	8.48	.568	.1128	.0498	.0630	----------	1:0.2:14.9
Wismer	Sept. 19, 1923	Nov. 27	8.04	3.11	11.15	.267	.1068	.0470	.0598	12.94	1:0.4:41.7
Worcester Pearmain	Aug. 14, 1923	Aug. 17	12.44	1.25	13.69	.328	.2930	.1470	.1460	14.85	1:0.89:41.1
Yellow Pippin	Nov. 4, 1924	Dec. 2	7.53	2.53	10.06	.317	.1180	.0412	.0768	----------	1:0.37:32.3
Yellow Skin	Oct. 11, 1922	Oct. 12	6.85	4.62	11.47	.558	.0754	.0254	.0500	----------	1:0.135:20.5
	Oct. 18, 1924	Oct. 23	7.99	2.34	10.33	.475	.0785	.0297	.0488	----------	1:0.165:21.7

[a] Fruit used grown at Virginia Agricultural Experiment Station, Blacksburg, Va.

[b] Slightly past prime condition for pressing when received at the laboratory.

That these assumptions hold in a general way is apparent from examination of the data. Fifty-two varieties were analyzed in 1923 and also in some other year (in two instances in two other years). In 40 of these, the 1923 analyses showed higher sugar content than did the other years. In 27 of the 52, total acidity is higher in 1923, and in 33 relative astringency is lower than in the other year or years. This is in good agreement with the results obtained with the group analyzed annually, for which 1923 was the year of maximum sugar and minimum relative astringency content. The year of minimum sugar content in the work with the large group was 1924. Thirty-eight varieties of this group were analyzed in that year and also in some other year or years. Thirty-two of these had lower sugar content, 25 had lower acid content, and 25 had higher relative astringency in 1924 than in the other year in which they were analyzed. Thirty-six varieties were analyzed in 1922 and also in some other year. In 26 of these cases there was also an analysis made in 1923, a year of maximum sugar content in the large group. In 18 of these 26 varieties sugar content was lower, and in 19 relative astringency was higher, in 1922 than in 1923. Twelve of the varieties analyzed in 1922 were also analyzed in 1924, which was in the case of the large group a year of lower sugar content than was 1922. In 9 of these the sugar content was higher, in 10 the acid content was higher, and in 10 the relative astringency was lower in 1922 than in 1924.

The analyses of 1922 and 1923 present one exception to the situation generally present, in that the acid content of the 1922 samples is not prevailingly lower than in the same varieties in 1923. While actually lower in 10 of the 26 cases, it is equal or higher in the remaining 16. Acid content thus fails to behave in agreement with sugar content in this instance. This emphasizes the fact that climatic conditions influence sugar and acidity in the same direction but not necessarily in the same degree; there is no relationship between the two constituents such that they must necessarily rise or fall strictly together.

With this exception, the results of the analyses conform, in the quantity and interrelations of the constituents under discussion, to expectations based upon the group studied consecutively over the period. The exceptions discoverable would appear to be of the frequency and character observable in all work involving large numbers of individuals, whether of one variety or of various botanical or horticultural varieties. It therefore appears to be justifiable to consider that any analysis here presented has somewhat greater meaning than can be attached to an isolated analysis unaccompanied by any information as to the conditions under which the material employed was grown. The years in which the analyses were made have been evaluated, for purposes of apple production, by ascertaining the composition of a large number of varieties which were found to behave in high degree as one. In so far as these analyses permit comparison of varieties with themselves in different years, the results accord with the behavior of the group. The group swung together through a range of chemical composition, its place within the range in any year being determined by the conditions of the year. In so far as internal evidence indicates, the varieties here dealt with behave in the same manner as the group. It appears justifiable to consider that the cases in which only a single analysis was made were mani-

festing the same general behavior, and that an analysis made in 1920 or 1924 presents a close approximation to the minimum sugar content and maximum astringent content to be encountered in the variety under normal cultural conditions at Washington; that an analysis in 1923 or 1921 may be regarded as near the maximum sugar and minimum astringency to be expected here; and that 1922 will show an absence of extremes, the results standing near the middle of the range for both constituents.

The acid-astringency-sugar ratio, that is, the ratio obtained by dividing the total astringency and the total sugar content by the titratable acidity, has been calculated for each analysis and the results are included in Table 2. The importance of this ratio has been pointed out elsewhere (7); it attempts to derive from the analytical data an expression of the character of the material as judged by the sense of taste. Since sugar and astringent materials are influenced in opposite directions as regards their quantity by a given set of climatic conditions, it follows that the ratio will vary from year to year with varying climatic environment. The absolute amount of variation in any one constituent may not be great, but the collective effect of the simultaneously occurring variations in opposite directions of two constituents which are concerned in determining palatability or dessert quality is large. It is responsible for the wide differences in palatability or appeal to the taste which are observable in the fruit or juice of the same variety from season to season. The acid-astringency-sugar ratio is an attempt to sum up in one expression the factors responsible for the collective effect upon the taste organs of the constituents which they perceive. It furnishes a sort of shorthand summary of an analysis, permitting much more convenient and rapid comparisons between analyses than is otherwise possible. Thus a glance at the ratios in Table 2 shows at once that the juice of Bigg was much more acid and astringent in 1924 than in 1923; that Bloomfield and Connett Sweet were more astringent and less sweet in 1922 than in 1923, that the juices of Crookstem were very closely similar in 1922, 1923, and 1924, while those of Carlough were much more astringent in 1922 than in 1924. Such comparisons made directly from the analytical data demand several separate mental calculations with resulting lack of clarity. While the acid-astringency-sugar ratio tells nothing as to the absolute amounts of any of these constituents, it states their quantitative relationship to one another, and it is this quantitative relationship which is perceived by the sense of taste and expressed as a verdict of "too sweet," "too acid," "just right," or similar phrases. It is certain that many of the chemical analyses of apples and other fruits in the literature fall short of conveying as adequate conceptions of the organoleptic quality, the appeal to the taste, as they would convey had data upon the astringency content been included. The inclusion of such data in analyses, with the conversion of the analytical results into the form of the acid-astringency-sugar ratio, will materially aid the reader in deriving a conception of the effect upon the taste organs from the analytical data.

SUMMARY

Data upon the chemical composition of the juices of 98 varieties of apples grown at the Arlington Experiment Farm, Rosslyn, Va., near Washington, D. C., in the years 1920 to 1924 are presented.

Two of the varieties were analyzed in 3 years, 63 in 2 years, and 33 in 1 year only. Analyses of 10 varieties of French cider apples grown at the Virginia Agricultural Experiment Station, Blacksburg, Va., 3 of which are also represented in the material grown at the Arlington farm, are also included in the tables. Many, perhaps most, of the 105 varieties concerned are not now represented by analyses in the literature.

The analytical results lend confirmation to the conclusion established by previous work that climatic conditions during the period of development and maturity produce consistent and sustained effects upon the chemical composition of the fruit of apple trees, the character of these effects being that of mass responses given by large groups of trees of dissimilar origin, adaptation to local conditions, and character of fruit.

Since the climatic conditions of the years in which the analyses were made are definitely known, and their specific effects upon the composition of their respective crops have been determined for large groups of varieties, any individual analysis has somewhat greater significance than would be the case were it not accompanied by such information.

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BACTERIAL HALO SPOT OF KUDZU CAUSED BY BACTERIUM PUERARIAE HEDGES[1]

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INTRODUCTION

The first appearance of the kudzu vine (*Pueraria thunbergiana*) in America is said to have been at the Philadelphia Centennial Exposition in 1876, where it was used to cover the Japanese tea houses. Once introduced it remained, these few plants having been left behind by the departing Japanese in the hands of a few fanciers of foreign plant oddities. In recent years, however, the vine has become of some economic importance in the South as a forage crop, a fact which has resulted in an increased interest in the problems connected with its culture and the diseases affecting it.

HISTORY OF THE DISEASE

In August, 1925, badly spotted leaves of kudzu (pl. 1, A, B, C, and E), collected August 27 at Cairo, Ga., by O. C. Boyd, of the Georgia State Board of Entomology, were received by the writer with the statement that the "infection is just now becoming prominent." A month later a second lot of material was received, with the information that the disease had spread very little in the fields during the time that had elapsed since the submitting of the first specimens. It had been found in another field, however. No general survey had been made. The weather had been unusually hot and dry. The disease proved to be bacterial. The parasite was isolated, and laboratory and greenhouse work was carried on through the winter in Washington.

In June, 1926, a badly diseased vine found June 3 at Sylvester, Ga., was received from Boyd. There were few, if any, sound leaves on the plant, and this, wrote Boyd, was typical of all vines in a 5-acre field planted with roots obtained from Florida. The owner had rooted out all the vines in this field, hoping to prevent the spreading of the disease to an adjoining 30 acres of kudzu planted with Georgia roots. At this time the latter field was free from disease with the exception of a few rows adjacent to the field of diseased Florida kudzu. Later in the summer, however, Boyd found the whole of the 30-acre field of Georgia kudzu very badly infected, the disease being present in a most virulent form.

In the latter part of June, 1926, the writer visited kudzu fields in the vicinity of Cairo, Ga., and Monticello, Tallahassee, and Gainesville, Fla.

Much of the disease was present in the Cairo, Ga., field from which the original material had been obtained the preceding August.

[1] Received for publication Nov. 12, 1927; issued May, 1928.

Three fields were visited which had been planted with roots from this field, one in March, 1925, and two in February or March, 1926, i. e., one the spring preceding and two the spring following the discovery of the disease in August, 1925. Many young infections were found on leaves and runners in all three of these fields. In one of the 1926 plantings the young foliage of some of the vines was thickly beset with small angular water-soaked spots (pl. 1, D), from which the kudzu organism was later isolated.

Little, if any, of the disease was observed in the other five fields visited in the vicinity of Cairo.

The disease was also found in the vicinity of Tallahassee, Fla., though to a minor extent. It was not observed either at Monticello or Gainesville, Fla.

GEOGRAPHICAL DISTRIBUTION

The writer has thus far observed this disease only in Georgia and Florida, but no extended survey has been made. In the summer of 1924 Clinton [2] reported in Connecticut a kudzu bean spot, of which he wrote: "New to State and probably to the United States; produced yellow spots on leaves exactly like wildfire of tobacco, but so far have failed to produce the disease; some spots are like the bacterial spot of bean." In 1926 dried specimens of this material were sent to the writer by Clinton for comparison. The disease appeared to be the same as that here described.

DESCRIPTION OF THE DISEASE

The disease forms conspicuous spots on the leaves and glistening brown streaks on the succulent young runners. The infections are stomatal and the youngest spots are angular and water-soaked. (Pl. 1, D.) Later the center becomes brown and is surrounded by an irregular translucent area. (Pl. 1, A, C, E.) Slightly older spots have in addition a wide yellow halo. (Pl. 1, B.) Finally the surrounding tissue becomes brown before the diseased area has withered and dried. The bacteria are abundant in the center of the spot, but in the halo they are present chiefly if not exclusively in the bundles, from the cut ends of which in free-hand sections they pour out in masses.

The wide yellow halo, from which the common name of the disease is derived, is strikingly like that in tobacco wildfire. It is much more striking when the spots on a leaf are comparatively few. When they are close together the halo is less in evidence or disappears altogether.

ISOLATION OF THE PARASITE

The parasite is easily isolated from fresh material or even from that which has been kept dry in the laboratory for three or four weeks. The only difficulty which the writer has thus far experienced in this regard was in isolating the organism during a long-continued period of excessive heat in Washington, when both diseased material (collected four weeks previously) and poured plates were

[2] CLINTON, G. P. KUDZU-BEAN. U. S. Dept. Agr., Bur. Plant Indus., Off. Plant Disease Survey, Plant Disease Rptr. Sup. 42:354. 1925. [Mimeographed.]

kept at the unusually high room temperature (about 34° C.). (See "Temperature relations," p. 427.)

The leaves will stand a treatment with $HgCl_2$ 1:1,000 for 30 to 45 seconds, but such sterilization is not necessary. Equally good results were obtained by careful washing, either rubbing between thumb and forefinger under the tap or dipping in alcohol and then passing through a series of two to five tubes of sterile tap water, allowing the pieces of tissue to remain a few minutes in each. The colonies appear in one to two days.

INOCULATION EXPERIMENTS ON KUDZU

Attempts to grow kudzu from seed in the greenhouse met with very little success during the winter of 1925–26. Most of the seeds failed to germinate, and those that did produced only very stunted plants, all but four of which soon died. These four survivors were kept for some time in the hope that they would begin to grow, and though they never produced more than a few leaves, they were finally inoculated, as they were the only kudzu plants available. They were sprayed in an inoculating cage in the greenhouse with a water suspension of a 2-day-old beef-agar culture of the kudzu organism. At the end of 24 hours, during which they were continually moist, the plants were removed from the inoculating cage. They were under observation for six weeks, but only one of the four ever showed any sign of infection. This plant had produced a few new leaves since inoculation. The other three had made no growth at all. From this one infected plant typical colonies of the kudzu organism were isolated. Parallel inoculations on Lima bean produced excellent infections, as hereinafter described.

During the summer of 1926 kudzu vines were very successfully grown from seed in the greenhouse, and on these excellent artificial infections were obtained in August. The following method was employed: A few drops from a thinly clouded 24-hour-old beef-broth culture were poured on both sides of young leaves, which were then rubbed lightly with a cotton plug and sprayed with a fine spray of sterile water. Some of the leaves were covered with paraffined bags, but no attempt was made to keep the other inoculated leaves moist for a number of hours. The plants were placed in the shade, however. The cultures used were from three different sources—the 100 per cent infected field in Sylvester, Ga., the Cairo field in which the disease was originally discovered, and a 1926 planting from the latter. All were from isolations made in June and July, 1926.

A week after inoculation there were excellent infections from all three strains, although the Sylvester organism seemed to be the most virulent. There were masses of very small, angular, water-soaked spots on the under surface of the leaf, in every way similar to those observed in the kudzu fields in June. On the upper surface the spots were brown. Some had large pale-green halos. The leaves in the paraffined bags were no more seriously infected than the others.

INOCULATION EXPERIMENTS ON LIMA BEAN

Owing to the difficulty experienced in growing kudzu satisfactorily in the greenhouse during the winter of 1925, all but one of the inoculation experiments carried on during that period were made on Lima

beans after a preliminary set of prick inoculations had shown that the kudzu organism was infectious to the Lima bean. Prick inoculations in stem and leaf blade resulted in a yellowing of the inoculated leaves, a reddening of the veins, and a subsequent wilting and shriveling. The organism was reisolated from such leaves.

Spray inoculations on Lima beans kept moist in inoculating cages for 24 hours after spraying with water suspensions of young beef-agar cultures, produced excellent infections in the greenhouse. In 8 to 10 days the leaves were covered with small irregular water-soaked spots. (Pl. 1, H, I.) By reflected light the whole spot appeared water-soaked. By transmitted light there was a pale-green halo with an inner red ring and a paler yellowish green or colorless center. Occasionally the center was red also. Four or five weeks after inoculation, the infected areas, much increased in size, were pale yellowish green or reddish brown, sometimes with a darker red border surrounded by a more or less yellowed area. Some of the veins in the infected areas were much reddened. In such an infected area covering about 8 sq. cm., bacteria were found in abundance five weeks after inoculation, and the kudzu organism was reisolated.

Excellent secondary infections appeared on pods (pl. 1, G) borne on Lima-bean plants inoculated by spraying when in blossom. Infections had been observed on the young leaves of these plants 11 days after inoculation. The spots on the pods were water-soaked and there was a white bacterial ooze. The older spots had reddish or yellowish centers and were considerably sunken. From both the white ooze and the water-soaked tissue the kudzu organism was reisolated.

Prick inoculations on green Lima-bean pods in a damp chamber produced water-soaked areas on both pods and seeds. (Pl. 1, F.) The first signs of infection were visible in two days. The small, tender, green seeds were especially susceptible.

The pathogenicity of the kudzu organism repeatedly isolated from artificial infections on Lima beans was proved by inoculation experiments.

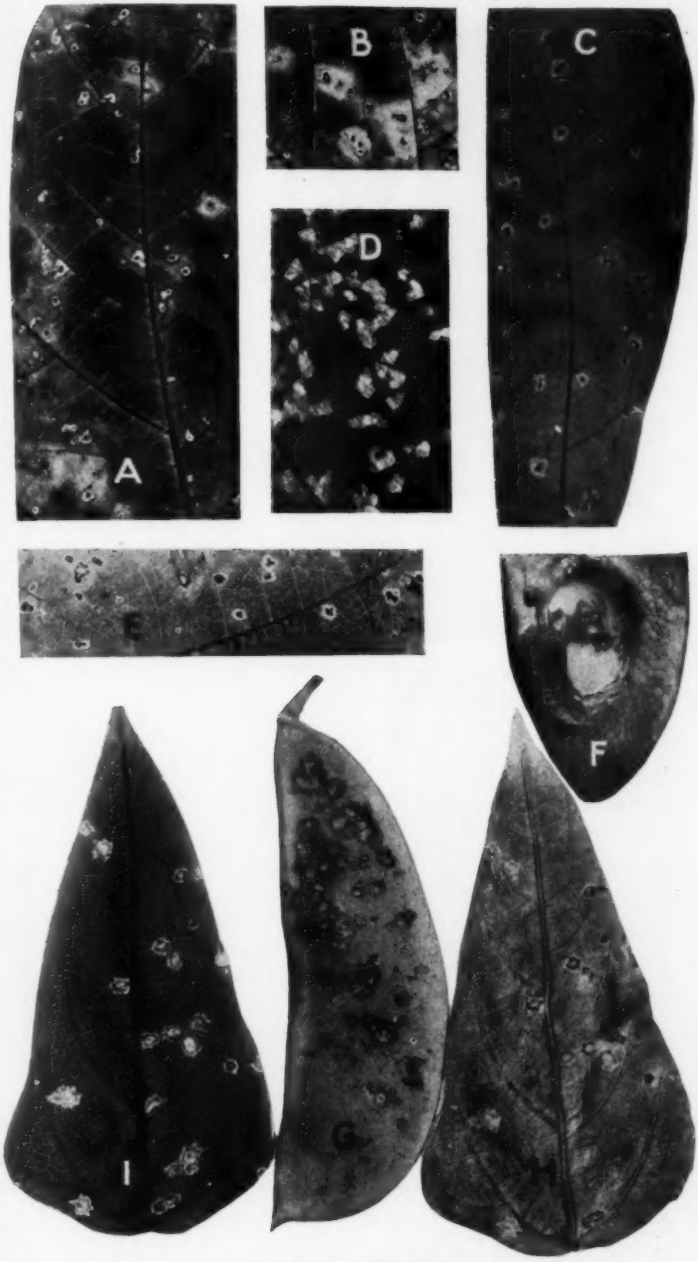
DISSEMINATION AND CONTROL OF THE DISEASE

No experiments in the dissemination and control of the disease have, as yet, been carried out, but there is some circumstantial evidence that the disease may be introduced into a new field by planting roots or runners from a field in which the disease is present (p. 420). The causative parasite, *Bacterium puerariae*,[3] is capable of long-continued growth at low temperatures (p. 427), and the writer believes that the organism is able to live over the winter in the dead leaves, bits of which may be left clinging to the transplanted roots and act as centers of infection in the spring. In practice no special attempt has been made to remove such débris before transferring the roots to a new field.

The planting of runners from an infected field is also a dangerous practice, as the succulent young stems are very susceptible and might

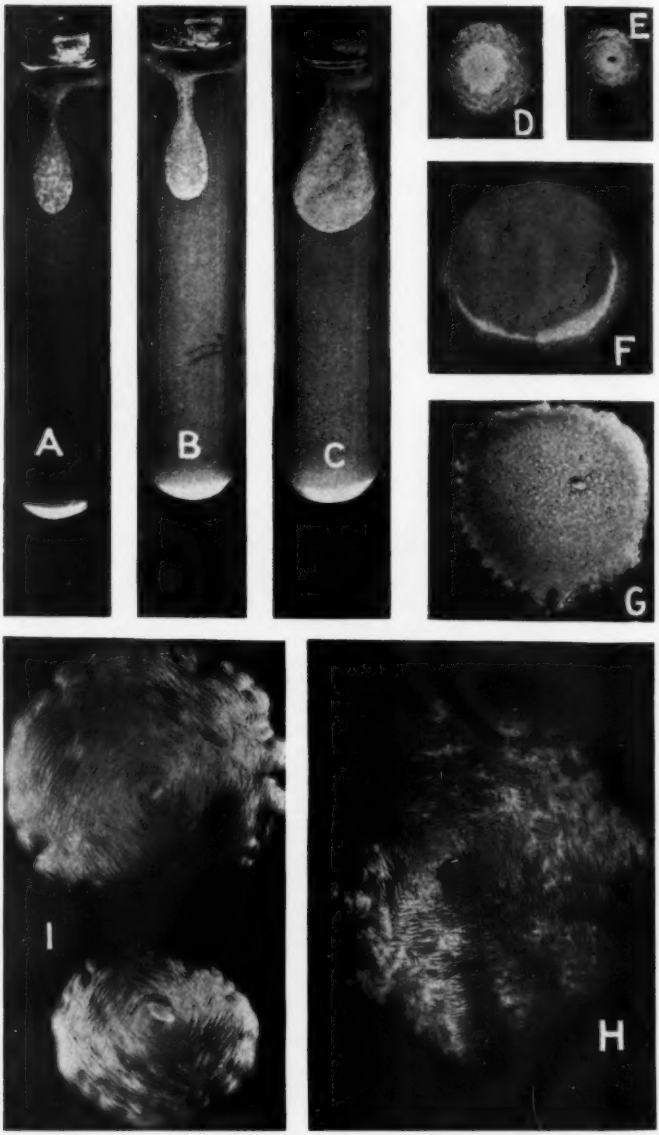
[3] An abstract of a preliminary paper presented before the American Phytopathological Society has appeared as follows:

HEDGES, F. BACTERIAL HALO SPOT OF KUDZU. (Abstract) Phytopathology 17: 48. 1927. (Through typographical error the name of the causal organism appears in this paper as *Bacterium pueriae* instead of *Bact. puerariae*.)



BACTERIAL HALO SPOT

A–E.—Natural infections on kudzu: A–C and E, older spots showing halo, × 1; D, early water-soaked stage photographed by transmitted light, × 5; E, photographed by transmitted light
F–I.—Artificial infections on Lima bean: F, infected seed, prick inoculations 9 days old; G, secondary infection on pod borne on plant inoculated by spraying when in blossom; H, spray inoculation 10 days old on young leaf, × 2; I, same leaf photographed by transmitted light



CULTURES OF BACTERIUM PUERARIAE

A.—Gelatin culture 5 days old. Saccate liquefaction. No liquefaction the fourth day. × 1
B.—Same, 24 hours later. × 1
C.—Same at end of 2 weeks. × 1
D, E.—Colonies on peptonized beef-infusion agar, 2 days old, showing white centers. × 10
F.—Same colony as D, 3 days old. × 10
G.—Colony growing next to the glass, 13 days old. × 10
H.—Colony 9 days old. × 10
I.—Colony 10 days old. × 8

serve as direct carriers of the disease. As the percentage of plants obtained from cuttings is very low, according to one of the oldest growers of this plant in America, there is a twofold reason for discontinuing the practice altogether.

In the present state of knowledge the writer can only recommend the use of well-established roots from which bits of dead leaves and runners have been removed. These should be selected from disease-free fields. As a further precaution the discontinuance of planting from runners is advisable.

CULTURAL CHARACTERS OF THE PARASITE

Cultural studies of *Bacterium puerariae* gave the results stated in the following paragraphs. All cultures were grown at room temperature unless otherwise stated.

BEEF-INFUSION (PEPTONIZED) AGAR PLATES.—Colonies appear in 1 to 2 days (21°–23° C.), and even when very young (2 to 3 days) show by oblique light the internal crosshatched markings or concentric striae which characterize them. (Pl. 2, D, E, H, I.) In occasional platings the young 2-day-old colonies have a very white center (pl. 2, D and E), which soon disappears, leaving a partial white ring, irregular in width, near the margin. (Pl. 2, F.) At first the colonies are round or approximately so, but later the margin becomes crenate. (Pl. 2, H and I.) This characteristic has been observed as early as the second day but is more commonly found in colonies a week or more old. When very much lobed the colonies become somewhat contoured. Colonies are bluish white, becoming somewhat opalescent, smooth, shining, and translucent. Young colonies are often very convex, older colonies almost flat; 3 to 6 mm. in diameter, sometimes in thinly sown plates, 9 to 10 mm. in diameter. No greening of the agar has ever been observed in plate cultures. Buried colonies are broadly fusiform, and those next to the glass are round and thin with coarsely granular centers and thinner regular or crenate margins. (Pl. 2, G.)

BEEF-INFUSION (PEPTONIZED) AGAR SLANTS, P_H 6.8–7.0.—Thin whitish filiform growth in 24 hours (21°–23° C.), flat, glistening, finely contoured, translucent, butyrous, little if any odor. The growth shows a tendency to become smoother as the cultures grow older. Growth is always rather scanty or moderate, never copious. Slight but distinct greening of the medium is usually visible in about 48 hours.

BEEF-INFUSION (PEPTONIZED) AGAR STABS, P_H 7.0.—Good whitish nailhead, scanty growth in upper part of stab. Slight but distinct green fluorescence usually noticeable in upper part of tube in about two days.

BEAN-PLANT AGAR.—Very scanty, barely visible growth in 24 hours. Thin, whitish, shining, somewhat contoured growth and slight green fluorescence in three days. The organism never made more than a scanty growth on this medium.

NUTRIENT (BEEF-INFUSION) GELATIN, P_H 7.2.—Slow saccate liquefaction of gelatin (pl. 2, A–C), beginning in 5 to 13 days (temperature varied widely, 10°–22° C.). In some cultures the liquefaction finally became stratiform. Complete liquefaction has never been observed, although the cultures have been under observation for three and one-half to seven and one-half months. Green fluorescence was produced in 2 to 4 days.

POTATO CYLINDERS (STEAMED).—Potato considerably grayed in 24 hours (21°–23° C.). Growth barely visible at this time; usually a moderate growth in 2 to 6 days, cream colored, wet shining, and smooth when thick enough to hide the irregularities in the surface of the potato. Sometimes there is only a scanty growth.

BEAN JUICE.—Moderately clouded in 24 hours. Slightly green fluorescence in three days.

BEEF-INFUSION (PEPTONIZED) BOUILLON, P_H 7.0–7.2.—Clouding in 24 hours. Faint rim and flocculent pellicle in older cultures; flocculence. Good growth in this medium, best near the surface. There is often a marked zoning, i. e., a heavy clouding in the uppermost part of the culture to the depth of about 1 cm., then a comparatively clear zone, and again good clouding in the lower half of the culture. Usually a slight blue-green fluorescence in about six days.

FERMI'S SOLUTION.—Clouding in 24 hours (23°–26° C.). Beautiful bluish green fluorescence in 6 to 14 days. A heavy pellicle is formed, which breaks up easily and is precipitated in flakes; wide white rim. An excellent medium for this organism.

LITMUS MILK.—No acid; alkali produced in three to five days. No coagulation; slow peptonization beginning in three to four weeks, not complete in two months. Litmus not reduced.

METHYLENE-BLUE MILK.—Only a trace of blue remaining in five days. Color slowly returned.

USCHINSKY'S SOLUTION.—Growth resembles that in Fermi's solution. Blue-green fluorescence, wide white rim, heavy pellicle breaking up easily into flakes.

DIASTASIC ACTION

To test the diastasic action of *Bacterium puerariae*, streak inoculations were made on beef-extract agar plus 0.2 per cent cornstarch in Petri dishes. *Bact. phaseoli*, which has a strong diastasic action, was used as a check. The seventh day a saturated solution of iodine in 50 per cent alcohol was poured into the plates. There was some diastasic action with all the six colonies of *Bact. puerariae* tested, but only two of them produced a clear zone entirely free of starch. This zone varied from 1 to 5 mm. in width and was clearly visible to the naked eye. When the plates inoculated with the other four colonies were examined with the binocular microscope it was observed that much of the starch had been consumed, although enough was left to make the zone blue. The amount of diastasic action produced in a given culture varied along the different portions of the streak and, strangely enough, did not appear to be correlated with the amount of growth. In the *Bact. phaseoli* checks there was a perfectly clear zone 2 to 3 cm. wide on either side of the streak in which no starch at all was observed even with the binocular microscope.

FERMENTATION OF SUGARS AND ALCOHOLS

ACID PRODUCTION

In the first series of fermentation experiments beef-extract agar (containing 0.5 per cent peptone, P_H 6.6) was used as the basic medium. To this was added 1 per cent of the sugar to be tested and brom cresol purple as an indicator.

Acid was produced from all but one (lactose) of the five sugars tested and from one of the two alcohols, as follows:

GLUCOSE.—Acid produced in 24 hours; acid throughout in two to three weeks. Temperature, 19°–24.5° C.

SACCHAROSE.—Acid produced in two days. Temperature, 20.5°–23.5° C.

GALACTOSE.—Acid produced in two days. Temperature, 21°–23° C.

LEVULOSE.—Acid produced in four days. Temperature, 21°–23° C.

LACTOSE.—No acid produced. Under observation five weeks. Temperature, 21.5°–25.5° C.

GLYCERIN.—Acid produced in four days. Temperature, 21°–23° C.

MANNIT.—No acid produced. Under observation 16 days. Temperature, 21°–23° C.

Good growth on glucose, saccharose, galactose, levulose, and glycerin. Poor growth on lactose and mannit.

A second series of experiments was made, using ammonium phosphate agar (peptone free) as a base. This medium, originally P_H 5.9, was adjusted to P_H 7.0 before the addition of the sugars and alcohols. The results were similar to those obtained with the beef-

extract base. Another sugar (maltose) was tested, however. Brom cresol purple was the indicator, as in the preceding series. The results were as follows:

GLUCOSE.—Acid produced in 2 days; acid throughout in 13 days. Temperature, 21°–23° C.
SACCHAROSE.—Acid produced in 2 days; acid throughout in 13 days. Temperature, 21°–23° C.
GALACTOSE.—Acid produced in 2 days; acid throughout in 13 days. Temperature, 21°–23° C.
LEVULOSE.—Acid produced in three days. Temperature, 21°–23° C.
LACTOSE.—No acid produced. Temperature, 21°–23° C.
MALTOSE.—No acid produced. Temperature, 21°–23° C.
GLYCERIN.—Trace of acid in two days. Some of the cultures were acid throughout in three days. Temperature, 21°–23° C.
MANNIT.—No acid produced.

As in the first series of experiments, good growth was correlated with acid production. There was excellent growth on glucose, saccharose, galactose, levulose, and glycerin and barely distinguishable growth on lactose, maltose, and mannit.

ALKALI PRODUCTION

Alkali production was studied on three sugars, using beef-extract agar (same stock as in the acid-production studies) with phenol red as a base, with the following results:

GLUCOSE.—Alkali produced in three or more days. Temperature, 19°–24.5° C.
SACCHAROSE.—Alkali slowly produced; none in two weeks, but alkaline throughout in seven weeks. Temperature, 20.5°–23.5° C.
LACTOSE.—Alkali produced. Temperature, 21.5°–25.5° C.

GAS PRODUCTION

No gas was produced from glucose, saccharose, lactose, or glycerin in ammonium phosphate agar stabs. Temperature, 22°–24° C.

NITRATE REDUCTION

The sulphanilic acid α-naphthylamine test described in the manual of the Society of American Bacteriologists was used, with *Bacillus phytophthorus* as a check. Tests were made at the end of 24 and 48 hours and 4, 7, and 10 days. There was no reduction of potassium nitrate to nitrite by *Bacterium puerariae*. Ammonia produced; no gas. Temperature 23° C. *Bacillus phytophthorus* used as a check, reduced the nitrate in 24 hours.

PRODUCTION OF AMMONIA, HYDROGEN SULPHIDE, AND INDOL

Cultures 48 hours old of *Bacterium puerariae* in peptone beef-extract broth, with and without potassium nitrate, showed the presence of ammonia when tested with Nessler's solution.

No hydrogen sulphide was produced in peptone beef-infusion broth P_H 7.0 and P_H 7.4. Strips of filter paper saturated with lead acetate solution and suspended in the neck of the tube served as the indicator. *Bacillus coli* and *Bacterium phaseoli* were used as checks.

The Gnezda oxalic-acid test for indol gave negative results with cultures of *Bacterium puerariae* in Dunham's solution made with peptone containing tryptophane. *Bacillus coli* was used as a check.

MINIMUM, OPTIMUM, AND MAXIMUM HYDROGEN-ION CONCENTRATION

The first series of studies to determine the minimum, optimum, and maximum P_H was made in beef-infusion broth with P_H reactions ranging in a series of short steps from 4.9 to 9.8. Growth occurred in 19 hours in P_H 5.6 to 8.8 (best in P_H 6.5). At the end of three days no additional tubes had clouded. In five days the P_H 9.0 was clouded, having become more acid and thus within the range of the organism. The P_H 5.0 never clouded (under observation 16 days).

A similar series was tested, substituting beef-extract broth for beef infusion. In this experiment the growth range of the organism was slightly extended. The P_H reaction ranged from 4.9 to 9.5. In 19 hours there was growth in P_H 5.5 to 9.1. In 43 hours P_H 9.4 was also clouded.

The optimum P_H in the beef-infusion broth is 6.5 to 7.0; in the beef-extract broth there seemed to be equally good growth at 5.5, 6.0, 6.4, and 6.8.

No green fluorescence was produced in beef-extract broth. In the beef-infusion broth most fluorescence was produced at P_H 8.0.

OXYGEN REQUIREMENTS

There was no evidence of anaerobic growth in shake cultures or stab cultures covered with 10 c. c. of melted agar. Good surface growth occurred in both types of cultures. Beef-extract agar was used.

FLUORESCENCE

Bacterium puerariae should probably be classed as a green fluorescent organism, although the greening produced in beef-infusion media might easily be overlooked. In early stages of growth it may usually be observed in the uppermost centimeter of both beef-infusion agar stabs and beef-infusion broth at about P_H 7.0 to 8.0. Later this small amount of greening becomes diffused and is not easily distinguishable. When first observed in the beef-infusion broth it is slightly bluish green. No fluorescence is noticeable in plate cultures. It is more marked in beef-infusion gelatin stabs than in beef-infusion agar. There is slight greening of bean juice and bean-plant agar.

No fluorescence has been observed in beef-extract broth or agar.

Fermi's and Uschinsky's solutions become beautifully blue-green fluorescent.

LONGEVITY

The organism is known to live at least four weeks in leaves kept dry in the laboratory at moderate room temperatures (about 21°–22° C.). It has been plated from such material and found to be pathogenic.

The vitality of the organism is much affected by high temperatures (see "Temperature relations," p. 427), and it is probable that many of the bacteria die rather quickly in the drying tissues during hot weather.

Cultures kept at room temperature were alive at the end of six weeks on beef-infusion agar slants in Fermi's solution and sterilized bean juice.

It is the experience of the writer that stock cultures in Fermi's solution and beef-infusion agar can be kept alive one and one-fourth to one and one-half years in the ice box at about 19° C., and beef-infusion agar cultures have been known to survive two years and four months at that temperature.

TEMPERATURE RELATIONS

A series of cultures in beef-infusion broth P_H 7.1 were tested at 10 different temperatures ranging from 2.5°–33° C.

In 19 hours growth had occurred from 13°–29° C. There was no growth at 2.5°, 7°, 8°, or 33°. The optimum growth in 24 hours was at 20°–23°. At the end of four days the best growth was at room temperature (average 22+° C.). Growth finally occurred at all the temperatures. All clouded at the lowest temperature (average 2.2+°) in 5 days (one tube in 43 hours, a second in 4 days).

Cultures were kept in the 2.2+° C. compartment all through the winter (November 22, 1926, to March 9, 1927), transfers being made from them from time to time to test their vitality. The temperature ranged from 1.5°–2.5° during this period. On March 9 they were still alive, though somewhat less vigorous, the transfers taking 2 days to cloud instead of 24 hours.

To recapitulate: The optimum temperature is 20°–23° C., the maximum temperature above 33°, the minimum below 2.5°, and long-continued growth is possible at about 2.5°.

The inhibiting effect of high temperatures was demonstrated by the inability of the writer to isolate *Bacterium puerariae* from infected leaves when the room temperature at which both the diseased material and the poured plates were kept averaged 34° C. It was first thought that the organism had been killed in the tissues by the high temperature that had prevailed throughout the four weeks following the collection of the halo spot, but this proved not to be the case, inasmuch as successful isolations were made from the same material when the plates were put into the ice box at a temperature of 15°–19° immediately after pouring and kept there for the duration of the experiment.

To test further, at this juncture, the comparative effect of high room temperatures and the lower ones of the ice box, two parallel sets of cultures were made in Fermi's solution. One was placed in the ice box, the other kept at room temperature. A fairly constant temperature of 18.5°–19° C. was maintained in the ice box. The room temperature was 31°–34.5°. The cultures grew slowly at both temperatures at first, but at the end of 10 days the difference between them was striking. Those at the high room temperatures were only very thinly clouded, while those at 19° were heavily clouded. Both were bluish green fluorescent.

THERMAL DEATH POINT

The behavior of *Bacterium puerariae* in thermal death-point experiments (10 minutes at 48°–65° C.) was very erratic, a given culture sometimes containing a few individuals with a thermal death point a number of degrees higher than that of the remainder. A transfer from one such culture resisted 63°, though others from the same tube were killed by 60° and 58° C. None resisted 65°. The thermal death point of the majority is still in doubt.

MORPHOLOGY

On beef-infusion agar the organism consists of rods occurring singly or in pairs. Chains and filaments are also formed. The rods are polar flagellate, having 1 to 3 flagella at one or both ends. Capsules not observed. In 24-hour-old cultures on peptone beef-infusion agar the rods measured 0.86 to 1.67 by 0.3 to 0.48 μ.

REACTION TO STAINS

Bacterium puerariae is easily stained with carbol fuchsin and the "aqueous-alcoholic" solutions of gentian violet and basic fuchsin. It is Gram negative and not acid fast. No capsules were demonstrated with Ribbert's capsule stain. The flagella stain readily with the Casares-Gil flagella stain.

BRIEF DESCRIPTION OF BACTERIUM PUERARIAE HEDGES

A short polar-flagellate noncapulate rod, 0.86 to 1.67 by 0.3 to 0.48 μ; chains present; endospores absent; Gram negative; not acid fast; producing "halo spot" on the kudzu vine (*Pueraria thunbergiana*), also pathogenic to Lima bean; strictly aerobic; liquefying gelatin; no reduction of nitrates to nitrites or gas production in nitrate media; ammonia produced; no production of hydrogen sulphide or indol; no chromogenesis on beef-extract bouillon or agar; slight but distinct blue-green fluorescence in beef-infusion (peptonized) bouillon and agar; marked greening of nutrient gelatin and beautiful blue-green fluorescence in Fermi's and Uschinsky's solutions; slight diastasic action; produces acid from glucose, saccharose, galactose, levulose, and glycerin, none from lactose, maltose, or mannit; no gas produced from glucose, saccharose, lactose, or glycerin; growth on peptonized beef-infusion agar in 24 hours moderate, whitish filiform, flat, glistening, finely contoured; colonies on peptonized beef-infusion agar bluish white or opalescent, round becoming crenate or lobed, smooth, shining, translucent, with internal crosshatchings or concentric striae; no acid or coagulation produced in milk; production of alkali and slow peptonization in milk; no reduction of litmus in litmus milk; steamed potato cylinders grayed, growth cream colored; optimum P_H is 6.5; growth range in peptonized beef infusion broth is P_H 5.6 to 8.8 (19 hours); optimum temperature for growth about 22° C.; maximum temperature above 33°; minimum temperature below 2.5°; thermal death point, see page 427.

SUMMARY

This paper describes a disease of kudzu vine. The disease is characterized by a conspicuous spotting of the leaves, the presence of wide yellow halos when the spots are not too close together, and glistening brown streaks on the succulent young runners.

Halo spot is known to occur in Georgia, Florida, and Connecticut.

From the spots an organism, *Bacterium puerariae* Hedges, has been isolated and its pathogenicity proved by pure-culture inoculations. Its biological characters are herein described.

Bacterium puerariae is also infectious to the Lima bean.

Examination of kudzu fields furnished very good circumstantial evidence that the disease is introduced into new areas by planting them with roots from diseased fields. The organism has been proved capable of long-continued growth at low temperatures, and the writer believes that it lives over the winter in the dead leaves, bits of which remain attached to the transplanted roots and act as centers of infection in the spring.

Roots for transplanting should be selected from disease-free fields. The use of cuttings should be avoided, as the succulent young runners are very susceptible to infection and might easily serve as direct carriers of the halo-spot disease.

CHANGES PRODUCED IN APPLES BY THE USE OF CLEANING AND OIL-COATING PROCESSES[1]

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INTRODUCTION

In the past two years it has become necessary in several of the Rocky Mountain and Pacific Coast States to employ cleaning methods for the purpose of removing excessive spray residue from apples after harvest. These methods are of two general types—dry brushing or wiping the fruit and washing it in chemical solutions. The latter method is the more effective and is coming into general use. The cleansing liquids employed are usually dilute solutions of either an acid or a base. Hydrochloric has been found to be the best acid at strengths varying from ⅓ to 3 per cent by weight of the acid, depending upon conditions. Of the basic solutions, sodium hydroxide or sodium carbonate are generally used in strengths similar to those given for hydrochloric acid. Disinfectants are sometimes added to the washing solutions. A discussion of these processes has been reported by Heald, Neller, and Overley,[2] and it suffices here to state that the hydrochloric-acid treatment is conducted in tanks with varying degrees of agitation, or under spray pipes from which the liquid, under moderate pressure, is directed upon the fruit. Alkaline solutions are similarly used, except that the liquid is generally heated to about 43° C. In both cases the fruit is washed in water before it is dried and packed. Fruit that has been treated with an alkali, such as sodium hydroxide, is given a light coating of a mineral oil-paraffin mixture to retard shriveling of the skin. The oil is generally applied by means of revolving brushes.

It is probable that these cleaning processes affect the physiological activities of the fruit, particularly when they are followed by the addition of a protective coating of oil. The purpose of the present paper is, by presenting a comparison of treated and untreated fruit, to show some of the effects produced by these treatments. The fruit used in the experiments was commercially harvested, cleaned, and treated in an irrigated section of central Washington.

EXPERIMENTAL METHODS

Special attention was given to the effects of the treatments upon the respiratory rates of the fruit. These were determined by measuring the evolution of carbon dioxide from duplicate or triplicate samples of from six to eight apples each. The maximum variation

[1] Received for publication Dec. 27, 1927; issued May, 1928. Published with the approval of the director of the Washington Agricultural Experiment Station as Scientific Paper No. 142, College of Agriculture and Experiment Station, State College of Washington.

[2] HEALD, F. D., NELLER, J. R., OVERLEY, F. L., and DANA, H. J. ARSENICAL SPRAY RESIDUE AND ITS REMOVAL FROM APPLES. Wash. Agr. Expt. Sta. Bul. 213, 56 p., illus. 1927.

between duplicate determinations was 9.68, the minimum 1.71, and the average 5.25 per cent.

By the method used, air was drawn through soda-lime towers to remove CO_2. It was next bubbled through a half-inch column of 10 per cent H_2SO_4, and then into desiccators containing the weighed fruits. The air was drawn out from the bottoms of the containers and through a series of CO_2 absorption towers containing 0.3 normal NaOH. Carbon dioxide was determined by precipitating the carbonate with $BaCl_2$ and titrating the excess of NaOH.

The air currents were drawn through the units at a fairly rapid rate for four hours each day. The determinations were made at laboratory temperatures, these being recorded on a thermograph. Although these temperatures fluctuated to a considerable extent, they do not introduce a variable factor into the comparative data obtained, since all the determinations of any given comparison were obtained at the same time and at the same temperature.

During the period in which respiration determinations were being made, duplicate samples of the same lots of apples were held in the same room to determine the loss in weight and moisture. These were stored unwrapped in open-top paper bags.

EFFECT OF BRUSHING AND WASHING ON RESPIRATION RATES AND LOSS IN WEIGHT

The first experiments were made to determine the effect of dry brushing upon the respiratory rate of the fruit. A rotating spiral-brush type of machine was used on Winesap apples that had been in cellar storage for about three and a half months. The rate of carbon dioxide evolution during the first six days after brushing was somewhat increased by this treatment, as is shown in Table 1. Where the dry brushing was preceded by an acid-spray treatment the respiration rate was 22 per cent greater than that of the untreated fruit.

TABLE 1.—*Respiration rates and loss in weight of dry-brushed and of acid-washed dry-brushed Winesap apples taken from cellar storage; experiments begun January 10, 1927* [a]

Treatment	Carbon dioxide per 100 gm. of fruit				Increase over untreated fruit	Loss in weight in 14 days
	Jan. 12	Jan. 14	Jan. 16	Total		
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*	*Per cent*	*Per cent*
Dry brushed	4.75	4.79	6.20	15.74	12.10	5.04
Sprayed with hydrochloric acid and dry brushed	5.49	5.45	6.19	17.13	22.00	5.83
Untreated	4.51	4.31	5.22	14.04	----------	2.93

[a] The data are the average of duplicate determinations.

Two months after the first group of experiments was begun some of the same lots of fruit were treated with 2 per cent solutions of hydrochloric acid and sodium hydroxide at 25° C. for 10 minutes with constant mild agitation but without brushing or wiping. The respiratory rate for the first six days after treatment was slightly less than that of the untreated fruit. (Table 2.)

TABLE 2.—*Respiration rates and loss in weight of Winesap apples taken from cellar storage and treated with sodium hydroxide or with hydrochloric acid; experiments begun March 11, 1926* [a]

Treatment	Carbon dioxide per 100 gm. of fruit				Decrease over untreated fruit	Loss in weight, Mar. 11 to 18
	Mar. 13	Mar. 15	Mar. 17	Total		
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*	*Per cent*	*Per cent*
2 per cent sodium hydroxide	10.55	7.02	6.60	24.17	2.18	4.12
2 per cent hydrochloric acid by weight	10.23	6.89	5.99	23.11	6.52	3.07
Untreated	10.82	7.21	6.68	24.71	----------	3.31

[a] The data are the average of duplicate determinations.

During the first seven days after treatment the loss in weight of fruit treated with hydrochloric acid was practically the same as that of the untreated fruit (Table 2), but there was a considerably increased loss where sodium hydroxide was used. In the previous work with fruit from this lot it may be noted (Table 1) that dry brushing caused weight to be lost at a much increased rate and that an additional treatment with an acid spray caused but little further loss. An acid treatment without brushing or wiping likewise failed to produce an appreciable effect on the rate of loss. (Table 2.) Robinson and Hartman [3] also found that an acid-bath treatment had little effect on loss of weight as compared with the loss from wiping or brushing, which apparently disturbs and removes some of the protective wax coating. Heald, Neller, and Overley have shown, however,[4] that a brushing treatment which causes a rapidly increased loss of weight at laboratory or room storage has practically no effect on loss of weight when the fruit is held in cold storage at 32° to 33° C.

EFFECT OF OILING ON RESPIRATION RATES, LOSS IN WEIGHT, AND MOISTURE CONTENT

A considerable portion of the northwestern apple crop is prepared for storage and marketing by being coated with a thin film of oil after it has been cleaned by dry brushing or with a solution containing sodium hydroxide. In the process followed in these experiments the oil used was a highly refined, highly viscous mineral grade and was mixed with paraffin wax. It was warmed sufficiently to dissolve the paraffin and was spread over the surface of the apples by means of revolving brushes.

On November 1 some newly harvested Winesap apples that had been dry brushed and oiled were put in cold storage, together with some of the same lot that had been dry brushed but not oiled. At the same time untreated fruit was also stored. Four months later samples were brought to the laboratory for respiration determinations. The results show that whereas the respiratory rates of the brushed and untreated fruits were practically the same, they were both over 40 per cent higher than that of the oiled fruit. (Table 3.) In 1924 Magness and Diehl reported [5] data on the respiration of Winesap

[3] ROBINSON, R. H., and HARTMAN, H. A PROGRESS REPORT ON THE REMOVAL OF SPRAY RESIDUE FROM APPLES AND PEARS. Oreg. Agr. Expt. Sta. Bul. 226, 46 p., illus. 1927.
[4] HEALD, F. D., NELLER, J. R., OVERLEY, F. L., and DANA, H. J. Op. cit.
[5] MAGNESS, J. R., and DIEHL, H. C. PHYSIOLOGICAL STUDIES ON APPLES IN STORAGE. Jour. Agr. Research 27: 1–38, illus. 1924.

apples which showed that when they were coated with mineral oil and with paraffin the evolution of carbon dioxide was reduced by about 40 per cent. Their results were similar to those of Table 3 and were obtained at approximately the same temperature as the present writer used. Magness and Diehl also made a study of the CO_2–O_2 respiratory ratio and found that there was little anaerobic respiration in apples thinly coated with oil and stored at a temperature of 18° C. or lower. At 26°, however, there was considerable anaerobic respiration, with a consequent development of poor flavor. It was pointed out that this tendency became more pronounced as the thickness of the oil coating was increased.

TABLE 3.—*Respiration rates, water content, and loss in weight of brushed and of oil-coated Winesap apples after four months in cold storage; experiments begun February 28, 1927* [a]

Treatment	Carbon dioxide per 100 grams of fruit				Increase over oiled fruit	Water content Mar. 22	Loss in weight Mar. 1–22
	Mar. 2	Mar. 4	Mar. 6	Total			
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*	*Per cent*	*Per cent*	*Per cent*
Brushed	11.61	9.60	7.00	28.21	44.8	84.23	4.83
Oil coated	7.52	6.42	5.54	19.48	----------	84.05	4.51
Untreated	11.10	8.50	7.68	27.28	40.1	84.00	5.29

[a] The data are the average of duplicate determinations.

Loss-in-weight determinations showed that the unoiled fruits of Table 3 lost weight but little faster than the oiled fruits. The moisture content of the brushed, oiled, and untreated fruits was about the same. Figure 1 shows the air temperature and relative humidity that prevailed during the progress of the above experiment.

On June 22, or after nearly eight months of cold storage, other samples from the same lots of Winesap apples were subjected to respiratory measurements. The unoiled apples continued to respire at a faster rate than the oiled, although to a lesser extent. The increases amounted to 16.8 and 18.3 per cent, respectively, for the brushed and untreated lots. (Table 4.)

TABLE 4.—*Respiration rates of brushed and of oil-coated Winesap apples after eight months in cold storage; experiments begun June 20, 1927* [a]

Treatment	Carbon dioxide per 100 grams of fruit			Increase over oiled fruit
	June 22	June 24	Total	
	Mgm.	*Mgm.*	*Mgm.*	*Per cent*
Brushed	12.00	12.30	24.30	16.8
Oil coated	11.24	9.56	20.80	----------
Untreated	12.24	12.37	24.61	18.3

[a] The data are the average of duplicate determinations. Water content and loss in weight were not determined.

The process of brushing without oiling had little effect upon the respiration of these stored Winesap apples, as the brushed fruit respired slightly faster after four months and slightly slower after eight months of cold storage. (Tables 3 and 4.) Brushing had likewise little effect upon losses of moisture and of weight.

Samples of Delicious apples were prepared for storage in the same manner as were the Winesaps and they were kept in the same cold-storage room. As seen in Table 5, oil had a similar retarding effect upon respiration. This retardation amounted to 33.4 and 46.7 per cent, respectively, of the CO_2 evolution from the brushed and untreated lots. There was but little difference in the loss in weight of the different lots, but the moisture content of the oiled fruit was slightly higher.

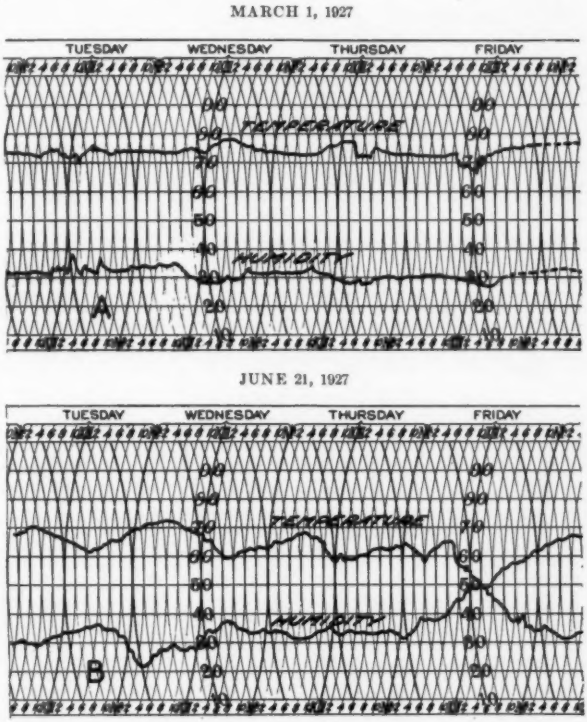

FIG. 1.—Temperature in degrees Fahrenheit and percentage of relative humidity that prevailed during respiration determinations of Winesap apples. A refers to the data in Table 3; B, to the data in Table 6

TABLE 5.—*Respiration rates, moisture content, and loss in weight of brushed and of oil-coated Delicious apples after four months in cold storage; experiments begun February 21, 1927* [a]

Treatment	Carbon dioxide per 100 grams of fruit				Increase over oiled fruit	Loss in weight, Feb. 21–Mar. 15	Moisture content, Mar. 15
	Feb. 23	Feb. 25	Feb. 27	Total			
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*	*Per cent*	*Per cent*	*Per cent*
Brushed	15.1	19.2	17.2	51.5	33.4	5.04	83.59
Oil coated	12.2	14.4	12.0	38.6	----------	4.87	84.54
Untreated	17.0	20.1	19.5	56.6	46.7	4.75	83.77

[a] The data are the average of duplicate determinations.

The respiratory rates of the Delicious apples were again determined after a period of eight months in cold storage. At that time (Table 6) the untreated fruit respired 43.28 per cent more carbon dioxide than the oil-coated fruit. Figure 2 gives the air-temperature and relative humidity record for the time during which the respiration and loss of weight determinations with Delicious apples were in progress. Although the fluctuations in temperature and humidity were considerable, they were the same for the compared treatments as given in the above discussion.

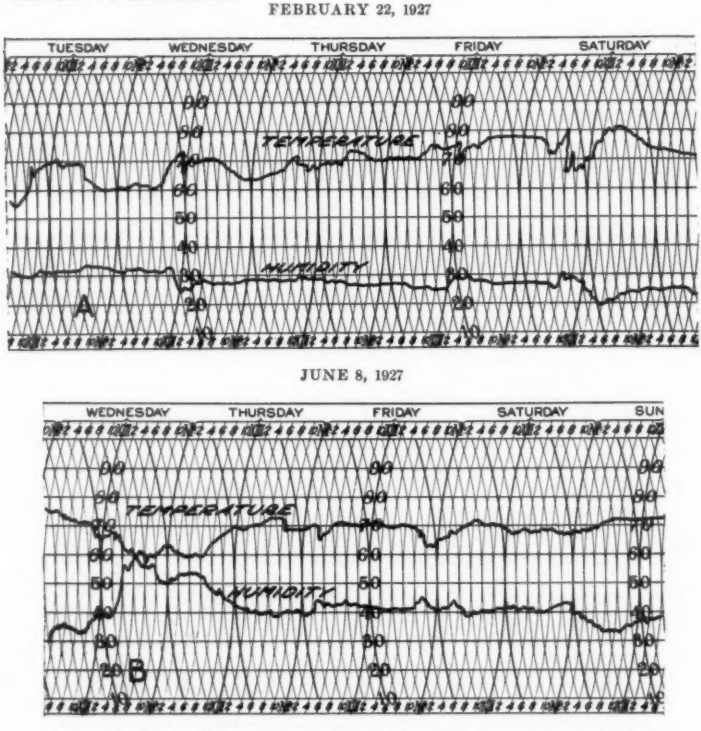

FIG. 2.—Temperature in degrees Fahrenheit and percentage of relative humidity that prevailed during respiration determinations of Delicious apples. A refers to the data in Table 5; B, to the data in Table 6

TABLE 6.—*Respiration rates and loss in weight of oil-coated Delicious apples after eight months in cold storage; experiments begun June 7, 1927* [a]

Treatment	Carbon dioxide per 100 grams of fruit			Increase over oiled fruit	Loss in weight, June 6–21
	June 9	June 12	Total		
	Mgm.	*Mgm.*	*Mgm.*	*Per cent*	*Per cent*
Oil coated	9.95	12.46	22.41		4.73
Untreated	14.23	17.87	32.10	43.28	5.54

[a] The data are the average of triplicate determinations. Water content not determined.

Dry brushing without oil coating slowed up the respiratory rate of Delicious apples as measured after four months of cold storage. (Table 5.) The data of Table 1 show a slight increase in the rate of respiration of Winesap apples as measured immediately after brushing. It is possible that the brushing process accelerated the respiration rate of the Delicious apples at first, resulting in less metabolic reserve when the respiration rates were determined four months later.

As shown in Tables 3 and 4, the effect of oiling was less apparent on the Winesap apples held eight months in cold storage than on those held four months. After eight months in storage the Winesap apples appeared very waxy, especially after warming up to room temperatures. This wax may have served to check respiration in much the same way as did the oil. The respiratory rate of the unoiled Delicious apples after eight months continued to be as much in excess of the oiled fruit as it was after four months of cold storage. The Delicious apples, of course, did not become nearly so waxy as the Winesap apples.

EFFECT OF OILING ON QUALITY OF FRUIT

It has been shown that coating apples with a film of oil causes a marked reduction in the respiratory rate of the fruit even after eight months of cold storage. The practice of oiling is employed when the fruit has been brushed or cleaned in an alkaline solution, the object being to give the fruit an oil-film protection equal or superior to the natural waxy protective coating of the untreated fruit. Since the experiments show that this cleaning and oil-coating process causes the respiration rate of the fruit to be considerably lowered, the question arises as to the effect of this practice upon the keeping and dessert quality of the fruit.

A comparison of apples by tasting or eating did not reveal any significant difference in dessert quality between treated and untreated fruit kept in storage for different lengths of time. When waxy apples like the Winesap variety, are brought to room temperature after a long period in cold storage, the excessive development of wax often makes it difficult to differentiate between the oiled and unoiled fruit even by visual examination. In most cases, however, the oil-coated fruit is easily recognized by its decreased luster and increased waxiness.

The oil-coated Delicious fruits could easily be distinguished from the unoiled, and the flavor was also slightly different. This change in flavor could not be declared either beneficial or detrimental.

DISCUSSION

In general, the experiments here reported have shown that the oil-coated fruit loses weight and shrinks somewhat less rapidly than the unoiled when brought into room or marketing temperature after long periods of cold storage. Since the oil-coated fruit also has a greater reserve of metabolic activity, as shown by respiration measurements, it is quite apparent that the practice of oil coating has a tendency to prolong the keeping quality of the fruit. In the fruit under test the dessert quality was neither noticeably benefited nor harmed. This fact indicates that the coating of oil as applied in these experiments was not sufficiently heavy to induce anaerobic respiration with its accompanying unpleasant flavors.

SUMMARY

Winesap and Delicious apples grown in an irrigated section of eastern Washington were placed in cold storage immediately after harvesting and cleaning. The cleaning methods used were (1) dry brushing, (2) dry brushing and oil coating, and (3) mild agitation in dilute hydrochloric acid without brushing or wiping. When the fruit to be tested had remained in cold storage for a certain time the rates of respiration and losses of moisture and of weight were determined at room or marketing temperature.

After four months in cold storage untreated Winesap apples respired CO_2 at a rate over 40 per cent faster than dry-brushed, oil-coated fruit. After eight months in cold storage the unoiled Winesap apples respired about 17 per cent faster.

Untreated Delicious apples respired about 46 per cent faster than the brushed, oil-coated lot after four months, and about 43 per cent faster after eight months in cold storage.

When exposed to room or marketing temperatures of about 20° C. and relative humidities of about 30 per cent of the maximum, the unoiled Delicious and Winesap apples lost weight at a moderately faster rate than the oil-coated fruit. The moisture content of the untreated was only slightly lower than that of the oil-treated apples.

A film of oil of the character and thickness used in the process employed in these experiments caused no significant difference in the dessert quality of the fruit after as much as eight months of cold storage. The oil coating tended to reduce the rate of shriveling and softening when the fruit was exposed to room or marketing temperatures.

Dipping in 2 per cent by weight of hydrochloric acid at 20° C. with mild agitation for 10 minutes had little effect upon the subsequent rates of either CO_2 respiration or loss in weight of Winesap apples. Dipping in 2 per cent sodium hydroxide under the same conditions affected respiration slightly and increased the rate of loss in weight.

The use of hydrochloric acid under the above conditions, except that contact was made with the fruit by means of jet sprays, resulted in unchanged rates of respiration and in no change in rate of loss in weight.

Brushing the fruit in either the dry or the wet cleaning process caused the respiration rate to be greater, and markedly increased the rate of loss in weight when the fruit was exposed to marketing temperatures immediately after the brushing treatments. When the fruit was held in cold storage for several weeks, beginning immediately after a brushing treatment, this effect of brushing became less marked.

CORRELATED STUDIES IN OATS OF THE INHERITANCE OF REACTION TO STEM RUST AND SMUTS AND OF OTHER DIFFERENTIAL CHARACTERS [1]

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INTRODUCTION

Stem rust caused by *Puccinia graminis avenae* Erikss. and Henn., and the smuts, *Ustilago avenae* (Pers.) Jens. and *U. levis* (K. & S.) Magn., are among the most destructive parasites of oats. Certain varieties of oats are resistant commonly to stem rust and others are immune from or resistant to the smuts. Stakman and others (*14*) [2] with stem rust and Reed (*12*) with the smuts have observed physiologic specialization. This complicates the problem of breeding resistant varieties, since it is difficult to control the physiologic forms of the disease under field conditions.

The stem-rust resistant parents used in these studies were segregates from crosses of White Russian with Minota or Victory. The White Russian variety has proved highly resistant to stem rust in the United States, and the rust-resistant segregates used as parents have been highly resistant at University Farm, St. Paul, Minn., for several years. They are resistant, therefore, to any or all forms of oat stem rust so far present in this locality. Black Mesdag was found by Reed (*11*) to be immune from attacks of both loose and covered smuts of oats, while Reed and Stanton (*13*) believed that resistance to both forms was dependent upon the same genetic factors. In the studies here reported no attempt has been made to use single physiologic forms of stem rust, and both loose and covered smuts have been present.

The purpose of the present paper is to present a study of the inheritance of reaction to stem rust and smuts in relation to other differential characters of the parents, more especially black versus nonblack glumes, presence of many or few hairs on the rachilla which supports the second kernel of the spikelet, and differences in awn development.

PREVIOUS STUDIES OF INHERITANCE

Stakman, Levine, and Bailey (*14*) have demonstrated instances of physiological specialization for stem rust of oats, *Puccinia graminis avenae* E. and H. Garber (*5*) and Griffee (*6*) obtained a segregation in F_2 of 3 resistant, 1 susceptible, using stem-rust inoculum collected in Minnesota. Later generations could be explained also on a single-factor pair basis. In these studies White Russian was used as the resistant parent.

[1] Received for publication Dec. 29, 1927; issued May, 1928. Published with the approval of the director as paper No. 743, Journal series of the Minnesota Agricultural Experiment Station.

[2] Reference is made by number (italic) to "Literature cited," p. 457.

Reed (*12*) observed physiological specialization in the smuts, although certain varieties appeared resistant to all races of smuts which he used. Black Mesdag proved immune from both covered smut, *Ustilago levis*, and loose smut, *U. avenae*.

Studies of reaction to smut in oats have demonstrated that immunity, resistance, and susceptibility are inherited characters. The difficulty of obtaining a heavy epidemic has made it impossible to determine the number and nature of the genetic factors involved without the use of more detailed studies than have been made so far. Wakabayashi (*15*) studied crosses of Red Rustproof, which is immune from covered smut, with Black Tartarian, which is susceptible. Immunity was dominant and several genetic factors were believed to be involved. Gaines (*4*) obtained similar results. Barney (*1*) suggested that reaction to loose smut in three different crosses could be explained upon a monohybrid, dihybrid, and trihybrid basis, respectively. The data presented, however, were not sufficient to prove the hypothesis. Reed and Stanton (*13*) in crosses of Fulghum, which is resistant to both loose and covered smut, with Swedish Select, which is susceptible, presented evidence to indicate that resistance to both forms was dependent upon the same genetic factors. Apparently both resistance and immunity are dominant over susceptibility. No cases of close linkage of the genetic factors which condition reaction to either stem rust or smuts and other differential characters have been reported.

Differences in awn development have been studied by numerous workers. Fraser (*3*) explained results which he obtained in crosses of Sixty Day and Burt on the basis of a single-factor pair for awning. His results agreed with those of Nilsson-Ehle (*10*) and Love and Craig (*9*), which indicate that the gene for yellow color of the glume inhibits awn development. Coffman, Parker, and Quisenberry (*2*) have reviewed most of the literature regarding the inheritance of weak and strong awns. In general, weak awns have proved dominant to strong awns and most of the results have been explained on a single main pair of genetic factors. Minor modifying factors are involved in some crosses, and environmental conditions may greatly modify the production of awns.

Black glumes have proved dominant or epistatic to other colors (*8*). In most cases black versus white have proved to be dependent upon a single-factor pair.

MATERIALS AND METHODS

The cultures used in these studies consisted of several apparently homozygous selections from crosses of Minota × White Russian or Victory × White Russian; Black Mesdag and F_1 to F_5 crosses between Black Mesdag and homozygous rust-resistant segregates from the White Russian crosses. Two of three strains used in the crosses with Black Mesdag have not been named. These are Minota × White Russian, Nursery Series Nos. II–18–37 and II–18–4. The Victory × White Russian selection, II–18–2, which was crossed with Black Mesdag, has been named "Anthony" and has been approved for distribution as the most promising stem-rust-resistant variety obtained so far. These three rust-resistant selections were obtained from the lines studied for reaction to rust by Garber (*5*) and by Griffee (*6*).

The crosses between selections II–18–2 and II–18–4 and Black Mesdag were studied primarily only for glume color and rust reaction, although a considerable number of lines with white glumes were studied for smut reaction as well.

The most intensive study of inheritance was made with the cross between the selection II–18–37 of the White Russian × Minota cross, and Black Mesdag. This selection produced open panicles, white glumes, none to a few weakly developed awns, and none to a few hairs on the pedicel or rachilla which is attached to the first or primary kernel of the spikelet and which supports the second kernel of the spikelet.

The Black Mesdag line was obtained from an oat classification nursery, the seed for which was obtained from Etheridge about 1920. At this time it was a pure-line selection. It has not been pedigreed recently and, except as mentioned later, appears homozygous. In contrast to the White Russian-Minota selection, it is susceptible to stem rust, immune from smut, produces black glumes, many strongly developed, geniculate awns, and many hairs on the rachilla of the second kernel of the spikelet. One plant of the 20 which were studied in 1923 deviated somewhat from the others. It was lacking in hairs on the rachilla, and the awns were more weakly developed than those of the other plants. It could have been obtained from a natural cross, and occasional natural crosses do occur in oats at University Farm, St. Paul (*7*). Such natural crosses are not frequent enough to seriously modify the ratios obtained in genetic experiments. They could explain an occasional aberrant type.

The studies of rust reaction were made by spraying the F_2 field cultures in 1923 with spore suspensions of stem rust, *Puccinia graminis avenae*, collected at University Farm, St. Paul, and cultured in the greenhouse. An unusually heavy infection was obtained in 1923 and separations into resistant and susceptible plants were made easily. Resistance is completely dominant over susceptibility. Resistant plants were tested further by growing cultures in the greenhouse from the plants which were resistant in the field and inoculating approximately 30 seedlings of each with cultures of stem rust.

Studies on reaction to smut were made by using smut material collected at University Farm, St. Paul. No attempt was made to control the type of smut used and both *Ustilago avenae* and *U. levis* undoubtedly were present. An examination of the material used as inoculum was made in 1925 and 1926. The percentage of smooth spores was approximately the same as that of echinulated spores, indicating that nearly equal amounts of covered and loose smut were used. While it would have been more desirable in certain ways to have made separate studies of these two smuts, the Black Mesdag parent was immune from both, and as a rule most oat varieties have reacted in a similar manner to both smuts (*11*, *12*). Reed and Stanton (*13*) in crosses of Fulghum with Swedish Select studied separately the reaction of F_3 to F_5 families to *U. avenae* and *U. levis* and concluded that the same genetic factors were responsible for reaction to both forms of smut.

Studies of reaction to smut are somewhat difficult as complete infection is seldom if ever obtained, and if it were obtained other

characters could not be studied at the same time. It seemed most important in these investigations to determine the proportionate number of immune lines without carefully studying the differences in degree of susceptibility in the susceptible and resistant lines. Individual F_2 plants were studied for rust reaction, pubescence on rachilla, and awn development. Random selections of approximately equal numbers of F_2 plants were selected in each of the four groups—black glumes resistant to rust, black susceptible, white resistant, white susceptible. Each such F_2 plant became the parent of an F_3 line. Twelve-foot rows were grown in F_3 and sown at the rate of 75 seeds per row. The seed was smutted by mixing an even teaspoonful of smut spores with the grain in each envelope. The glumes were not removed from the kernels, as it seemed more desirable to use large numbers and test the immune lines for several years than to obtain more refined data on the susceptible lines. Lines which were susceptible in F_3 were not tested again. All immune lines in F_3 were again tested in F_4. Such lines were harvested in bulk in F_3, and 2 gm. of seed mixed with smut spores were used for each F_4 row. All immune lines in F_4 were again studied in F_5.

The F_1 plants from which the F_2 material originated were grown near Washington, D. C., in a greenhouse of the United States Department of Agriculture, and only the threshed grains were available; consequently, no data were taken on this F_1. The Washington greenhouse material was grown and harvested under the direction of T. R. Stanton, agronomist in charge of oat investigations of the Office of Cereal Crops and Diseases. Stanton's cooperation made possible the saving of a crop season by growing the F_1 the same year that the cross was made. Several F_1 plants were grown at University Farm, St. Paul, the same season as the F_2. The F_1 and F_2 were practically free from smuts.

Correlated studies of reaction to rust and smut and color of glume, awn development, and pubescence on rachilla of the second kernel of the spikelet were made by correlating the differential characters of the F_2 plants with the F_2 and F_3 reaction to rust and the classification of smut reaction based upon the F_3 to F_5 data.

INHERITANCE OF DIFFERENTIAL CHARACTERS

The mode of inheritance of the differential characters, black versus white grain color, stem-rust resistance versus susceptibility, smut reaction weak versus strong awns, and few versus many hairs on the rachilla will be discussed, and following this a study of linkage or correlation of the characters will be made.

COLOR OF GLUMES

The F_1 glumes were brownish black, a little lighter in color than the black parent and shading over to a light brownish color, depending apparently on the degree of maturity. There is some variation in a homozygous black variety, however, and no reliable differentiation into homozygous and heterozygous black glumed plants seems to be possible.

The results obtained in the first group of experiments are given in Table 1. The color of kernel was found to be dependent on a single-factor difference in these crosses, the results approximating a 3 : 1 ratio.

TABLE 1.—*Number of black and white glumed plants in F_2 from crosses between Black Mesdag and three white-glumed strains*

Cross	Number observed		Number calculated		Total number of individuals	Deviation	Probable error	Deviation/P. E.
	Black-glumed plants	White-glumed plants	Black-glumed plants	White-glumed plants				
(White Russian×Victory) II-18-2× Black Mesdag	635	199	625.5	208.5	834	9.5	±8.43	1.13
(Minota×White Russian) II-18-37× Black Mesdag	2,907	1,028	2,951.2	983.8	3,935	44.2	±18.33	2.41
(Minota×White Russian) II-18-4× Black Mesdag	1,069	352	1,065.7	355.3	1,421	3.3	±10.98	.30
Total	4,611	1,579	4,642.5	1,547.5	6,190	31.5	±22.98	1.37

The greatest deviation of observed from expected numbers in a single F_2 group of crosses is 2.41 times the probable error, and for all F_2 plants the deviation is 1.37 times the probable error.

There were found occasionally a few small and unripe plants and their color was somewhat doubtful. This may have caused slight errors in the classification, but as a whole the two characters pigmented and nonpigmented glumes are easily distinguished and the results are in good agreement with expectation.

RESISTANCE TO STEM RUST

The resistance of the hybrid strains used as parents in the crosses here concerned originated from White Russian by the selection of homozygous resistant plants from crosses of a strain of this variety with Victory or Minota.

It appeared probable that also in the present crosses resistance might prove to be dominant and dependent on a single differential factor, as has been found in previous studies in which White Russian was one of the parents (*5*, *6*). But there was, of course, a possibility that Black Mesdag differed from the varieties Victory and Minota in one or more factors, fundamental for development of resistance.

The data obtained from the F_2 generation of crosses between Black Mesdag and the resistant strains mentioned are given in Table 2. Results are quite similar for the three crosses, and the segregation approximates a 3:1 or monohybrid ratio. The greatest deviation was 1.52 times the probable error for a single group and 1.15 times the probable error for the total number of individuals.

TABLE 2.—*Number of resistant and susceptible plants in F_2 from crosses between Black Mesdag and three white-glumed rust-resistant strains*

Cross	Number observed		Number calculated		Total number of individuals	Deviation	Probable error	Deviation/P. E.
	Resistant plants	Susceptible plants	Resistant plants	Susceptible plants				
(White Russian×Victory) II-18-2× Black Mesdag	625	209	625.5	208.5	834	0.5	8.43	0.06
(Minota×White Russian) II-18-37× Black Mesdag	2,942	993	2,951.3	983.7	3,935	9.3	18.33	.51
Minota×White Russian) II-18-4× Black Mesdag	1,049	372	1,065.7	355.3	1,421	16.7	10.98	1.52
Total	4,616	1,574	4,642.5	1,547.5	6,190	26.5	22.98	1.15

Practically all the white resistant plants were tested in the greenhouse during the fall of 1923 for rust reaction. From each F_2 plant which was tested, 20 to 25 seeds were planted in 4-inch pots. The seedlings were inoculated by spraying the plants with a fine spray of water and brushing the seedlings lightly with rusted plants of the susceptible variety Victory (*6*).

The rust used was transferred to the susceptible seedlings in the greenhouse from rusted plants grown in the oat nursery. The chief aim of the greenhouse work was to determine which F_2 plants were homozygous for rust resistance, and therefore no very accurate data on number of resistant and susceptible seedlings were taken. The plants were counted in an early stage before the rust was very well developed. All pots which showed a few susceptible seedlings were thrown away immediately after the data were taken, while the pots having only resistant seedlings were kept for a longer time. As a rule, however, the plants could be classified fairly accurately about eight days after inoculation. The results from 705 segregating F_2 families were 10,575 resistant: 3,378 susceptible plants. The deviation from a 3:1 ratio on the basis of numbers was 110, and the probable error was 34.5 individuals. Deviation P. E. = 3.188. When the above-mentioned facts are taken into consideration, the results are in fairly good agreement with expectation, and they give additional evidence in support of the theory of a single-factor difference between the resistant and susceptible parents. On the same factor basis, a 2:1 ratio of heterozygous resistant to homozygous resistant F_2 plants is expected. The actual results obtained are given in Table 3. The deviation of obtained from expected results is 1.9 times the probable error. The greatest deviation was in the first group of crosses (White Russian × Victory) II–18–2 × Black Mesdag, but in this case the deviation is only 2.24 times the probable error. A deviation as great as this is expected to occur on the basis of random sample once in every six or seven trials.

TABLE 3.—*Number of homozygous and heterozygous F_2 plants in the group white resistant from crosses between Black Mesdag and three white rust-resistant strains*

Cross	Number observed		Number calculated		Total number of individuals	Deviation	Probable error	Deviation/P. E.
	Homozygous plants	Heterozygous plants	Homozygous plants	Heterozygous plants				
(White Russian × Victory) II–18–2 × Black Mesdag	53	81	44.7	89.3	134	8.3	3.7	2.24
(Minota × White Russian) II–18–37 × Black Mesdag	238	468	235.3	470.7	706	2.7	8.5	.32
(Minota × White Russian) II–18–4 × Black Mesdag	91	156	82.3	164.7	247	8.7	5.0	1.74
Total	382	705	362.3	724.7	1,087	19.7	10.5	1.88

The reliability of the classification for rust resistance is of interest. There was quite a heavy rust epidemic in the oat nursery, which undoubtedly made it comparatively easy to classify the plants. Of about 1,090 plants, classified as white-seeded rust-resistant, which were tested in the F_3 generation, there were 5 plants which, by later

test, were found to be homozygous for susceptibility. As these 5 constitute less than 0.5 per cent of the total number tested, it will be seen that the character rust resistance can be considered just as dependable as any other plant character. About 120 white-seeded susceptible F_2 plants were tested in the greenhouse. Of these, 3 were without much doubt classified wrongly, as they segregated for rust resistance.

NUMBER OF HAIRS ON RACHILLA

For each parent, as well as each hybrid plant, six spikelets were used in the determination of hairiness. Two spikelets from the lower middle and upper part of the main panicle of each plant were studied. The hairs were counted under a binocular. Counts could be made accurately when a few or a medium number of hairs were present, but when the number was as high as 20 accurate counts were difficult. The average number of hairs on the rachilla which supports the second kernel of the spikelet was determined by obtaining an average for the six counts made from the panicle selected to represent each individual plant.

The results obtained are for random selections of F_2 parent plants within the four groups, black glumes resistant to stem rust, black susceptible, white resistant, and white susceptible. The parents, F_1 and F_2 generations were grown in 1923. The data obtained are given in Table 4.

TABLE 4.—*Average number of hairs on rachilla in Black Mesdag, rust-resistant selections, and F_1 and F_2 crosses of Black Mesdag with three rust-resistant selections*

Parents or crosses	Number of plants in groups based on number of hairs on rachilla				
	0-7	8-15	16-23	24-31	32-39
Black Mesdag	1	3	14	2	
White Russian×Victory II-18-2	20				
II-18-2×Black Mesdag F_1	11	1			
Black rust resistant F_2	208	33	26	2	1
Black rust susceptible F_2	116	29	9	6	
White resistant F_2	116	21	9	1	
White susceptible F_2	43	4	2		
White Russian×Minota II-18-37	30				
II-18-37×Black Mesdag F_1	15				
Black resistant F_2	132	21	6	1	
White susceptible F_2	115	19	4		1
White resistant F_2	139	16	1	1	
White susceptible F_2	128	9	6	1	
White Russian×Minota II-18-4	25				
II-18-4×Black Mesdag F_1	4	2			
Black resistant F_2	87	22	23	3	2
Black susceptible F_2	99	35	16	3	
White resistant F_2	96	31	14	6	
White susceptible F_2	71	22	3		

The segregation for color of glumes and rust reaction was on a single-factor basis in both cases. To obtain an accurate idea of the segregation for hairs on the rachilla, it was necessary to determine whether there was a linkage between rust reaction or glume color and numbers of hairs on rachilla. In the blacks, the ratio of hair group 0–7 to the total numbers in the other groups was 2.5 : 1,

while in the whites the ratio was 2 to 1. In the resistant and susceptible groups similar ratios for numbers of individuals in group 0–7 to the numbers in the remainder of the groups was 2.4 and 2.3 : 1, respectively.

The tendency of a correlation between high number of hairs on rachilla and black glume color makes a correction necessary before studying segregation for hairs on rachilla. Within each cross the data for the white segregates were calculated on the basis of a total of one-third as many white-glumed segregates as black. The segregation for hairs on the rachilla on this basis is presented in Table 5.

TABLE 5.—*Segregation for hairs on rachilla on the basis of one-third as many white as black glumed segregates*

Cross	Color of glume	Number of plants in groups based on number of hairs on rachilla					
		0–7	8–15	16–23	24–31	32–39	Total
II–18–2×Black Mesdag F_2	Black	324	62	35	8	1	430
II–18–2×Black Mesdag F_2	White	116	18	8	1	------	143
II–18–2×Black Mesdag F_1	Black and white	440	80	43	9	1	573
II–18–37×Black Mesdag F_2	Black	247	40	10	1	1	299
II–18–37×Black Mesdag F_2	White	89	8	2	1	------	100
II–18–37×Black Mesdag F_1	Black and white	336	48	12	2	1	399
II–18–4×Black Mesdag F_2	Black	186	57	39	6	2	290
II–18–4×Black Mesdag F_2	White	67	21	7	2	------	97
II–18–4×Black Mesdag F_1	Black and white	253	78	46	8	2	387

It is apparent from Table 4 that there is a dominance of few hairs over many. In the F_1 of cross of II–18–2×Black Mesdag 1 plant out of 12 was in group 8–15. Accordingly, on a single-factor pair basis, one-twelfth of the F_2 plants in group 8–15 were considered to belong to the dominant group. In the cross II–18–37×Black Mesdag no F_1 plants were in group 8–15. In the F_1 of II–18–4×Black Mesdag, 2 out of 6 plants were placed in group 8–15. The classification was corrected on the basis of the breeding behavior of F_1, and in the first cross one-twelfth of the plants in hair group 8–15 were considered to be F_1 types and of low hair number, while one-third of the plants in group 8–15 of cross II–18–4×Black Mesdag were considered to be F_1 types. The classification after this correction is as follows:

Cross	Hair group		Deviation from 3:1	Probable error for 3:1	Deviation/P. E.
	Few	Many			
II–18–2×Black Mesdag F_2	490	136	21	7.31	2.9
II–18–37×Black Mesdag F_2	513	76	71	7.09	10.0
II–18–4×Black Mesdag F_2	390	143	10	6.74	1.5

Two of the crosses could be satisfactorily explained on the basis of one main factor pair with a dominance of few hairs over many hairs, while in the cross of II–18–37×Black Mesdag the deviation from a 3:1 ratio is large.

To determine the genetic accuracy of the classification in F_2 several lines were grown in F_3 and studied for number of hairs on rachilla. The data for these F_3 lines are given in Table 6.

TABLE 6.—*F_3 breeding behavior for hairs on rachilla of certain F_2 plants in cross of II–18–37 × Black Mesdag*

Parent or F_2 cross	Average number of hairs on parent	Number of plants in groups, based on number of hairs on rachilla					
		0–7	8–15	16–23	24–31	32–39	Above
Black Mesdag		8	10	6	1		
II–18–37		25					
69–133 F_3	0.0	24	1				
-50 F_3	.0	25					
-113 F_3	.5	22	1	1			
-330 F_3	.7	17	6				
-47 F_3	1.0	20	4	1			
-1 F_3	2.8	23	2				
-5 F_3	4.2	23	2				
-31 F_3	6.3	10	6	6	1		
-46 F_3	8.5	19	2	5			
-7 F_3	11.3	11	5	6	3		
-250 F_3	12.8	3	4	4	8	4	2
-200 F_3	14.5	12	9	2	1		
-145 F_3	14.7	13	5	5	1	1	
-1550 F_3	14.3	7	11	4	2		1
-219 F_3	15.5	4	9	4	3	3	2
-1477 F_3	15.8	11	8	5	1		
-1536 F_3	16.2	5	7	3	5	4	1
-141 F_3	19.3	7	5	5	5	1	
-1470 F_3	21.3		2	8	6	5	4
-500 F_3	24.2		4	2	3	10	6
-12 F_3	26.1	3	2	2	2	10	6

The White Russian-Minota selection II–18–37 again bred true for few hairs and Black Mesdag proved more variable than in the preceding year, producing 8 out of 25 plants in the group 0–7. There was a general tendency for F_2 plants with few hairs to produce progeny with a low hair number, and F_2 plants with higher number of hairs on rachilla to produce progeny with a higher number. Some lines bred true for 0–7 hairs on the rachilla while some lines produced more hairs on the rachilla than the Black Mesdag parent.

AWN DEVELOPMENT

Inheritance of awn development was studied in a cross of Minota × White Russian II–18–37 with Black Mesdag.

Black Mesdag has a rather strong awn on the lower grain in the spikelet, but lacks awns entirely on the second grain.

The awn, as usually found on the first grain, is rather thick and long, with a dark basal portion which is twisted to the left from the base. This dark portion is brownish black and mostly uniform in color but is sometimes striped by light-colored bands. The twisting and coloring extend usually about one-third of the whole length of the awns. The geniculation, or bending of the awns, seems to be caused directly by the twisting, which ceases suddenly at the bend. The degree of geniculation varies considerably from almost a right angle to a scarcely perceptible bend and seems to be proportionate to the degree of twisting and to the strength of the awn. There is much variation in the geniculation and strength of awns between different spikelets

and panicles on a single plant. The average percentage of awned spikelets in Black Mesdag was found to be 91.9, varying in 18 plants from 73.1 to 100 per cent.

Strain II–18–37 produced an average percentage of 2.1 awned spikelets, as determined by an examination of 30 plants. Only 7 of the 30 plants, however, produced any awns.

The groups for awn development were differentiated on the basis of percentage of awned spikelets. They were placed in five groups on this basis—Group 1, 81–100 per cent (the awning percentage similar to Black Mesdag); Group 2, 61–80 per cent; Group 3, 41–60 per cent; Group 4, 21 to 40 per cent; Group 5, 0–20 per cent (the awning percentage similar to White Russian×Minota II–18–37).

The F_1 generation was intermediate both as to percentage and development of awns. Data on the parents and the F_1 and F_2 generations were compared. (See Table 7.)

TABLE 7.—*Inheritance of percentage of awned spikelets in a cross of Black Mesdag with a selection of White Russian×Minota II–18–37*

Parent or cross	Number of plants in groups as indicated [a]				
	Group 1	Group 2	Group 3	Group 4	Group 5
Black Mesdag	17	1			
II–18–37					30
Black Mesdag×II–18–37 F_1	2	6	3	1	3
Black Mesdag×II–18–37 F_2	66	70	58	44	140

[a] The groups were classified according to percentage of awned spikelets as follows: Group 1=81–100 per cent of spikelets awned; Group 2=61–80 per cent of spikelets awned; Gruop 3=41–60 per cent of spikelets awned; Group 4=21–40 per cent of spikelets awned; Group 5=0–20 per cent of spikelets awned.

The F_1 was as variable as the F_2 and gave all classes for percentage of awned spikelets. There was a large difference between the development of awns of the F_1 and F_2, which can be demonstrated by a classification on the basis of geniculation and percentage of awned spikelets.

The classification of the parents and of the F_1 and F_2 generation is given in Table 8.

TABLE 8.—*Geniculation and percentage of awned spikelets in a cross of Black Mesdag with a selection of White Russian×Minota II–18–37*

Parent or cross	Number of plants in groups as indicated [a]						
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
Black Mesdag	17	1					
II–18–37						23	7
Black Mesdag×II–18–37 F_1				6	6	3	1
Black Mesdag×II–18–37 F_2	66	21	12	57	58	105	59

[a] The groups were classified as follows: Group 1=geniculate, 67–100 per cent of spikelets awned; Group 2=geniculate, 34–66 per cent of spikelets awned; Group 3=geniculate, 1–33 per cent of spikelets awned; Group 4=nongeniculate, 67–100 per cent of spikelets awned; Group 5=nongeniculate, 34–66 per cent of spikelets awned; Group 6=nongeniculate, 1–33 per cent of spikelets awned; Group 7=no spikelets awned.

Plants in Groups 1 to 3 contained geniculate awns, and the F_1 showed a dominance of nongeniculate over geniculate, with some variability in the percentage of awned spikelets. There was a ratio in the F_2 of 99 plants in Groups 1 to 3 to 279 with weak or no awns. Fifty-nine F_2 plants produced no awns on the spikelet examined.

To determine the complexity of inheritance of the character, certain F_2 plants were selected and their F_3 progeny classified into seven groups for awn production, as in Table 9. It will be remembered that Groups 1 to 3 produce geniculate awns, Groups 4 to 6 nongeniculate awns, and that Group 7 produced no awns. The results of this study are presented in Table 9.

F_2 plants with 0 to few awns bred true or tended to breed true for this character in F_3. Eighteen of the twenty-nine nongeniculate F_2 plants produced progeny with some geniculate plants in F_3, although nongeniculate habit was produced in much greater numbers than geniculate. Of the F_2 geniculate plants which were tested by growing progeny in F_3, only 2 out of 11 tended to breed relatively true for geniculation. These two bred relatively true also for heavy percentage of awned spikelets.

TABLE 9.—*Classification of F_2 plants and F_3 progeny on the basis of number and geniculation of awns*

Classification of F_2 parent plant	Number of plants in F_3 progeny in groups as indicated [a]							Classification of F_2 parent plant	Number of plants in F_3 progeny in groups as indicated [a]						
	1	2	3	4	5	6	7		1	2	3	4	5	6	7
0 per cent						1	24	N, 55 per cent		1			8	11	5
Do						6	19	N, 58 per cent	1			1	2	19	7
Do					2	17	6	N, 60 per cent	2			2	6	15	
Do					6	11	8	N, 64 per cent	10	1		4	8	2	
Do					4	4	17	Do	2	2		5	8	7	1
N, 3 per cent [b]						3	22	Do	1				9	13	2
N, 5 per cent					1	8	16	N, 69 per cent	1			6	8	7	3
Do				1	4	6	14	N, 75 per cent	3			6	11	5	
N, 8 per cent		1			3	17	4	N, 79 per cent				1	6	18	
Do					1	14	10	N, 85 per cent				8	8	7	2
N, 11 per cent	3	3			3	13	3	N, 88 per cent	5			9	10	1	
N, 17 per cent	1	2		2	7	13	1	G, 20 per cent [b]	3	1		7	8	6	
N, 18 per cent				1	5	12	7	G, 31 per cent					9	12	4
N, 26 per cent		1			3	19	2	G, 29 per cent		4	1	1	4	13	2
N, 34 per cent			1	1	8	15		G, 48 per cent	1	3		1	8	11	1
N, 40 per cent					2	19	4	G, 52 per cent			1		12	12	
N, 41 per cent	2				2	19	2	G, 64 per cent	2	3		2	7	9	2
N, 44 per cent	2				8	14	1	G, 56 per cent	2	4			6	11	2
N, 46 per cent		3		2	8	10	2	G, 68 per cent		4	1	1	7	11	1
N, 48 per cent					2	21	2	G, 75 per cent				10	12	3	
N, 49 per cent					4	18	3	G, 98 per cent	21	1		3			
N, 52 per cent				2	19	4		G, 100 per cent	20	4			1		
N, 54 per cent		1		2	11	10	1								

[a] The groups were classified as follows: Group 1=geniculate, 67–100 per cent of spikelets awned; Group 2=geniculate, 34–66 per cent of spikelets awned; Group 3=geniculate, 1–33 per cent of spikelets awned; Group 4=nongeniculate, 67–100 per cent of spikelets awned; Group 5=nongeniculate, 34–66 per cent of spikelets awned; Group 6=nongeniculate, 1–33 per cent of spikelets awned; Group 7, no spikelets awned.

[b] N=nongeniculate; G=geniculate.

The lack of agreement between F_2 condition for awns and F_3 breeding behavior indicates that environmental conditions probably exert a rather marked influence upon type. It would appear relatively easy, however, to obtain weak-awned types from such a cross, but the genetics of awn development appear to be a more difficult problem. Without doubt several genetic factors are involved.

SMUT REACTION IN CROSS OF (WHITE RUSSIAN×MINOTA) II–18–37 WITH BLACK MESDAG

Random selections of F_2 plants were made from the four groups—black, susceptible to stem rust; black, resistant to stem rust; white, susceptible; and white, resistant. Approximately 100 F_3 lines were grown from each group. The Minota-White Russian susceptible parent was grown about every 20 rows and the Black Mesdag only once.

The grain was smutted before being sown. There was no infection in Black Mesdag. The infection in the various lines, of which approximately 75 plants of each were grown, was determined by counting the number of infected plants and the number of panicles infected and noninfected in each. The percentage of infected plants was multiplied by the average percentage of infected panicles of the plants which gave some infection. The lines which were not infected in F_3 in 1924 were harvested in bulk and tested in F_4. If they were again free from smut they were grown again in F_5. Lines with no infection were considered immune.

The lines of the smut-susceptible parent gave the following percentages of infection in 1924: 3.2; 4.6; 5.7; 6.4; 7.1; 7.9; 8.3; 8.4; 10.0; 10.8; 11.2; 12.4; 13.4; 15.0; 17.1. Immunity to smut in oats is dominant to susceptibility. If the same factors are involved, the susceptible F_3 lines should be infected as severely as the Minota × White Russian parent. If only a single-factor pair is involved, the heterozygous lines should be expected to contain one-fourth as much infection on an average as the susceptible parent. One-fourth of 3.2 is 0.8, while one-fourth of 17.1 is 4.3, and one-fourth of 15.0 is 3.8. On this basis, on an average, 2 out of every 3 F_3 lines which are included in infection groups 3.2 to 4.3 should be heterozygous for smut and 1 should be homozygous for smut susceptibility. The separation in each group was made on this basis.

The detailed separation of the F_3 hybrids with infection percentages between 3.2 and 4.3 is as follows: There were 11 F_3 lines in groups for smut infection 3.2–4.3 grown from F_2 plants classified as black susceptible to rust. These were classed as susceptible and heterozygous for smut reaction as follows:

Four susceptible (*S*), with infection percentages 3.7; 4.0; 3.9; 4.3.

Seven heterozygous (*H1*), with infection percentages 3.4; 3.2; 3.2; 3.7; 3.3; 3.4; 3.7.

Eight F_3 lines produced from F_2 black-glumed plants resistant to rust were placed in susceptible and heterozygous smut classes on the same basis:

3 susceptible, with infection percentages 4.2; 4.1; 4.1.

5 heterozygous, with infection percentages 3.5; 4.0; 3.5; 3.4; 3.4.

Six F_3 lines produced from F_2 white-glumed plants susceptible to rust were classed as follows:

2 susceptible, with infection percentages 4.3; 4.0.

4 heterozygous, with infection percentages 3.8; 3.6; 3.4; 3.9.

Five F_3 lines produced from F_2 white-glumed plants, resistant to rust, were classified for smut as follows:

2 susceptible, 4.1; 4.1.

3 heterozygous, 3.4; 3.3; 4.0.

The classification of F_2 plants on the basis of F_3 to F_5 breeding behavior (Table 10) was made as follows:

S=as susceptible in 1924 as II–18–37 Minota×White Russian, percentage of infection 4.4 or more.
H1=heterozygous in 1924 with one-fourth as much smut, on an average, as the smut-susceptible parent. (The 30 lines with infection percentages 3.2 to 4.3 were distributed in these groups, 11 in *S* and the remainder in *H1*, as has been explained.)
H2=0 infection in 1924, heterozygous with approximately one-fourth as much infection in 1925 as the susceptible parent.
H3=heterozygous in 1924 with lower infection than 8 smutted panicles.
H4=0 infection in 1924; about 10 panicles infected in 1925.
R=0 in 1924, 1 to 4 panicles in 1925 or 1926.
I=no infection in 1924 to 1926.

TABLE 10.—*Classification of the F_3 to F_5 progeny of four groups of F_2 plants on the basis of smut reaction*

F_2 parent plant	Number of plants in classes for smut reaction						
	S	H1	H2	H3	H4	R	I
Black, resistant to rust	17	32	9	4	2	7	8
Black, susceptible to rust	21	36	8	7	3	5	6
White, resistant to rust	19	37	4	2	5	13	20
White, susceptible to rust	29	44	8	6	2	11	13
Total	86	149	29	19	12	36	47

The results of these studies prove that it is very easy to obtain immune lines from a cross of immune and susceptible. The differentiation between different classes of homozygous, susceptible, and heterozygous families is very difficult with a character like reaction to smut, which is not completely expressed in the progeny. The results indicate that there are separate factors which differentiate immunity and resistance, for numerous lines of Black Mesdag have been grown and no infection has been obtained. Lines which produce only a few infected panicles may be resistant. Susceptible and heterozygous lines unless extensively studied are difficult of separation.

Results similar to those obtained in these studies could be explained by two pairs of genetic factors, *II* and *RR* for immunity and resistance, respectively, located in Black Mesdag. *I* might be considered to be epistatic to *R*. It is impossible, however, to determine the exact genetic condition in these studies.

CORRELATION BETWEEN COLOR OF GLUMES AND OTHER CHARACTERS

COLOR OF GLUMES AND RUST REACTION

The parents of the crosses differed in several characters which furnished an opportunity to study linkage relations. A classification for the characters resistance versus susceptibility and glume color is given in Table II. Resistance versus susceptibility and black versus white glume color are each dependent on a single genetic factor pair, and these two pairs of factors appear to be independently inherited.

TABLE 11.—*Correlation between glume color classes and reaction to rust in the F_2 generation of three crosses between Black Mesdag and rust-resistant segregates from previous White Russian crosses*

Cross	Black resistant plants	Black susceptible plants	White resistant plants	White susceptible plants	Total number of individuals	X^2	P
(White Russian×Victory) II–18–2×Black Mesdag, plant row No. 68:							
Obtained	477	158	148	51	834	.....	Close to 1.
Calculated	469	156.5	156.5	52	834	0.632	
(Minota×White Russian) II–18–37×Black Mesdag, plant row No. 69:							
Obtained	2,182	725	760	268	3,935	.....	.....
Calculated	2,213	738	738	246	3,935	3.286	0.3544
(Minota×White Russian) II–18–4×Black Mesdag, plant row No. 70:							
Obtained	781	288	268	84	1,421	.....	.....
Calculated	799	266.5	266.5	89	1,421	2.430	.4947
All F_2 groups, plant row Nos. 68–70:							
Obtained	3,440	1,171	1,176	403	6,190	.....	.....
Calculated	3,482	1,160.5	1,160.5	387	6,190	1.463	.6954

For plant row 68, X^2 is only 0.632 and P consequently very close to 1. For plant row 69, X^2 is somewhat higher, that is, 3.286, and P is 0.3544. For plant row 70, $X^2=2.430$ and $P=0.4947$, and for the whole F_2 population $X^2=1.463$ and $P=0.6954$. Resistance versus susceptibility and black versus white glume color appear to be independently inherited.

COLOR OF GLUMES AND HAIRS ON RACHILLA

Two of the crosses, II–18–2 and II–18–4×Black Mesdag, could be satisfactorily explained on the basis of a single genetic factor pair for hairs on rachilla with nearly complete dominance of few over many hairs. It will be remembered that the Black Mesdag parent produces many hairs and that the white-glumed parents produce few hairs on the rachilla. The results given in Table 12 are on the basis of one-third as many white as black glumed segregates.

TABLE 12.—*Correlation between glume color and hairs on rachilla in F_2 crosses of a black-glumed many-haired variety with white-glumed few-haired strains*

Cross	Number of black-glumed plants having—		Number of white-glumed plants having—	
	Few hairs	Many hairs	Few hairs	Many hairs
II–18–2×Black Mesdag	329	101	117	26
II–18–4×Black Mesdag	206	85	55	14
Total	535	186	172	40

There is apparently a lower percentage of white-glumed plants with many hairs on rachilla than black glumed, which indicates a possible slight genetic linkage between the factors for glume color and hairs on the rachilla. Comparing the results obtained for the two crosses with a 9 : 3 : 3 : 1 ratio on the basis of X^2 test of goodness

of fit, a value for P of 0.0858 was obtained. This means that on the basis of random sampling a deviation as great as the one obtained might be expected 858 times in 10,000 trials, or 1 in 11.7. This is not a wide deviation from expectation but here is a slight tendency for a linkage relation. Cross II–18–37 × Black Mesdag which deviated widely from a 3 : 1 ratio also produced more black-glumed many-haired segregates than white glumed. The percentage of many-haired segregates in the black and white glumed classes in this cross was 17.3 and 13.3 respectively. The results can be explained by the hypothesis of a loose linkage between the factors conditioning many versus few hairs and the factor pair for glume color.

COLOR OF GLUMES AND AWN DEVELOPMENT

The black and the white glumed F_2 plants were classified for awn development into seven groups. These were:

Group 1, geniculate awns, 67–100 per cent of spikelets awned.
Group 2, geniculate awns, 34–66 per cent of spikelets awned.
Group 3, geniculate awns, 1–33 per cent of spikelets awned.
Group 4, nongeniculate awns, 67–100 per cent of spikelets awned.
Group 5, nongeniculate awns, 34–66 per cent of spikelets awned.
Group 6, nongeniculate awns, 1–33 per cent of spikelets awned.
Group 7, no awns.

Black Mesdag was placed in Groups 1 and 2 and the few awned parents in Groups 6 and 7. The black and white glumed plants were placed in the seven awning classes as shown in Table 13:

TABLE 13.—*Classification for awn development of black and white glumed plants*

Color of glumes	Number of plants in groups for awn development							
	1	2	3	4	5	6	7	Total
Black	27	9	6	23	29	45	26	165
White	39	12	6	24	29	60	33	203

The double X^2 [3] test of goodness of fit was used to determine whether the two groups could be regarded as random samples drawn from the same population. A calculated X^2 of 1.98 was obtained and a P of 0.9210, which indicates that the two samples could reasonably be considered random samples drawn from the same population. The results indicate no correlation or linkage between the factors for awn development and those for glume color.

COLOR OF GLUMES AND SMUT REACTION

The material was classified for smut reaction on the basis of F_3 to F_5 breeding behavior. The color of glumes of F_2 plants was correlated with the breeding behavior for smut reaction. It will be remembered that the various lines were placed in several classes for smut reaction as follows: *S*, homozygous for smut susceptibility; *H1*

[3] The formula used was $X^2 = S\left\{\frac{NN'\left(\frac{fp}{N}-\frac{fp'}{N'}\right)^2}{fp+fp'}\right\}$, where fp = frequency distribution of one sample, fp' = the frequency distribution of the other; $S(fp) = N$ or total of one, $S(fp') = N'$ or total of other. $\frac{fp}{N}$, $\frac{fp'}{N'} = fp$ and fp', respectively, in percentage.

to *H4*, classes segregating for smut reaction and representing a progressive decrease in smut observed in classes *H1* to *H4*; *R*, resistant with only slight infection; *I*, immune from smut. The Black Mesdag parent was smut immune and the selections from the White Russian crosses used as the other parent were susceptible. The classification for color of glumes and smut reaction is given in Table 14.

TABLE 14.—*Classification of black and white glumed F_2 plants in relation to F_3 to F_5 breeding behavior of their progeny for smut reaction*

Color of glumes	Number of plants in groups for smut reaction						
	S	H1	H2	H3	H4	R	I
Black	38	68	17	11	5	12	14
White	48	81	12	8	7	24	33
Total	86	149	29	19	12	36	47

The double X^2 test was used to determine whether similar smut reaction groups were obtained for the black and white segregates. A calculated X^2 of 9.03 was obtained with a P of 0.1721. On this basis a worse agreement might be expected on the basis of random sampling 17 out of 100 trials. Probably therefore the segregation for smut reaction in the black and white glumed classes is of a similar nature.

CORRELATION BETWEEN RUST REACTION AND OTHER CHARACTERS

RUST REACTION AND HAIRS ON RACHILLA

Studies of correlation between reaction to stem rust and number of hairs on rachilla were made for the crosses of II–18–2 and II–18–4 with Black Mesdag which approached a monohybrid segregation for few versus many hairs. The results given in Table 15 are calculated on the basis of one-third as many susceptible as resistant plants, the classification for few versus many hairs being made as previously described.

It will be noted from the results that the agreement is good with a 9 : 3 : 3 : 1 ratio, which indicates that rust reaction and hairs on the rachilla of the second spikelet are independently inherited.

TABLE 15.—*Classification for reaction to rust and hairs on rachilla in the cross of Black Mesdag, a rust-susceptible, hairy-rachilla variety, with rust-resistant, few hairs on rachilla segregates of previous crosses of White Russian with Victory and Minota*

Cross	Number of resistant plants having—		Number of susceptible plants having—	
	Few hairs	Many hairs	Few hairs	Many hairs
II–18–2×Black Mesdag	162.7	48.0	53.0	17.2
II–18–4×Black Mesdag	141.6	61.0	48.9	18.6
Total	304.3	109.0	101.9	35.8

RUST REACTION AND AWN DEVELOPMENT

F_2 plants resistant and susceptible to stem rust were selected at random from each of the four groups—black resistant, black susceptible, white resistant, white susceptible. As there was no apparent linkage between black versus white and groups for awn development, the resistant and susceptible plants can be compared directly without correction for the fact that selection of black and white glumed plants was made. The relation between rust reaction and awn development is given in Table 16.

TABLE 16.—*Correlation between rust reaction and awn development in the F_2 generation*

Rust reaction	Number of plants in groups for awn development						
	1	2	3	4	5	6	7
Resistant	26	12	4	24	31	51	31
Susceptible	40	9	8	33	27	54	28
Total	66	21	12	57	58	105	59

On the basis of the double X^2 test the value of X^2 was 5.4 and the value of P was 0.4956.

RUST REACTION AND SMUT REACTION

F_2 plants were placed in two groups on the basis of reaction to rust—i. e., resistant and susceptible. F_3 to F_5 progeny selected at random from the four F_2 groups, black resistant, white resistant, black susceptible, white susceptible, were classified for smut reaction. As similar numbers of black resistant, and white resistant, black susceptible, and white susceptible plants were selected the smut reaction can be compared directly for the two groups for rust reaction. (See Table 17.)

TABLE 17.—*Correlated inheritance for rust and smut reaction*

F^2 plants for rust reaction	Number of plants in groups for F_3 to F_5 progeny for smut reaction				
	S	H1 and H2	H3 and H4	R	I
Resistant	36	82	13	20	28
Susceptible	50	96	18	16	19

The probability that these represent random samples from the same population was determined by the double X^2 test. $X^2 = 5.2$ and $P = 0.2697$. No correlation between rust and smut reaction was obtained.

CORRELATION BETWEEN HAIRS ON RACHILLA AND OTHER CHARACTERS

HAIRS ON RACHILLA AND AWN DEVELOPMENT

Black Mesdag produced many hairs on the rachilla and the rust-resistant parents produced few. Similarly, Black Mesdag produced many awns and the rust-resistant parents few. In order to determine whether there was an association between the characters, development of awns and rachilla hairs, the F_2 data from the cross of II–18–37 × Black Mesdag were used and the correlation between the two groups of characters determined. The coefficient of contingency was used as a measure of correlation. (See Table 18.) The probable error of this coefficient was calculated from the formula P. E. of C_1 or mean square contingency $= 2 \times 0.6745 \frac{1-C^2}{\sqrt{N}}$. The calculated value of $C_1 = 0.1867 \pm 0.0670$. This indicates little or no correlation, and therefore segregation for awn development and hairs on rachilla appears to be independent.

TABLE 18.—*Correlation between the development of awns and of hairs on the rachilla in the F_2 generation of II–18–37 × Black Mesdag*

Classes for hairs on rachilla	Number of plants in groups for awn development							
	1	2	3	4	5	6	7	Total
0–7	57	17	9	43	44	88	53	311
8–15	7	2	3	12	10	12	4	50
16–23	2			2	4	5	1	14
24–31		2					1	3
Total	66	21	12	57	58	105	59	378

HAIRS ON RACHILLA AND SMUT REACTION

The cross was between Black Mesdag, a smut-immune variety, which produces pubescence on the rachilla which supports the second kernel of the spikelet, and smut susceptible, weakly pubescent segregates of a previous White Russian cross. The F_2 generation of the cross of II–18–37 × Black Mesdag was used to determine the correlation, if any, between smut reaction and production of hairs on the rachilla. (See Table 19.)

The calculated coefficient of contingency for these results was 0.0435 ± 0.0692, which indicates that smut reaction and hairs on rachilla are independent in inheritance.

TABLE 19.—*Correlation between number of hairs on the rachilla which supports the second kernel of the spikelet of F_2 generation plants and smut reaction, as determined from their F_3 to F_5 generation progeny*

Classes for hairs on rachilla	Number of plants in smut-reaction classes					
	I	R	H3 and H4	H1 and H2	S	Total
0–7	40	30	28	150	63	311
8–15	6	3	2	20	19	50
16–23	1	2	1	6	4	14
24–31		1		2		3
Total	47	36	31	178	86	378

CORRELATION BETWEEN AWN DEVELOPMENT AND SMUT REACTION

The Black Mesdag or immune parent produces many strongly developed awns while the smut-susceptible Minota × White Russian parent, II–18–37, produces a few weakly developed awns. F_2-generation plants were classified into seven groups for development and geniculation of awns as has been described.

A correlation table for awn development in F_2 and smut reaction as determined from F_3 to F_5 data is presented in Table 20.

TABLE 20.—*Correlation between awn development of F_2 plants and smut reaction of their F_3 to F_5 progeny*

Smut reaction	Number of plants in F_2 groups for awn development							
	1	2	3	4	5	6	7	Total
I	9	3	2	5	6	18	4	47
R	9	2	1	5	3	7	9	36
H3 and H4	7	3	1	5	6	8	1	31
H1 and H2	31	12	7	21	27	51	29	178
S	10	1	1	21	16	21	16	86
Total	66	21	12	57	58	105	59	378

The coefficient of contingency for awn development and smut reaction was $C_1 = 0.1121 \pm 0.0685$. No close association or linkage is indicated.

SUMMARY

A correlated study of the mode of inheritance of reaction to black stem rust, *Puccinia graminis avenae*, and loose and covered smuts, *Ustilago avenae* and *U. levis*, in relation to other differential characters was made.

The rust-resistant parents were segregates from previous crosses of White Russian with Victory or Minota. Three selections were used, "Anthony," which was selected from a cross of White Russian × Victory and which bore the Nursery Series Number II–18–2, and two unnamed selections from crosses of White Russian with Minota which bore the Nursery Series No. II–18–4 and II–18–37. Nursery Series No. II–18–37 was used most extensively in the studies. These three selections produced white glumes, weak awns, few to no hairs on the second kernel of the spikelet and were susceptible to smut. The smut-immune parent, Black Mesdag, produced black glumes, strongly developed, geniculate awns, many hairs on the rachilla of the second spikelet, and was rust susceptible.

Black versus white glumes and rust resistance versus susceptibility gave close approximations to 3 : 1 segregations with black dominant over white and rust resistance dominant over susceptibility.

Segregation for number of hairs on the rachilla of the second spikelet agreed well with a 3 : 1 ratio in F_2 of few to many hairs on the spikelet for the crosses of II–18–2 and II–18–4 with Black Mesdag. The cross of II–18–37 and Black Mesdag produced a greater proportion of few-haired segregates than would be expected on the basis of a 3 : 1 ratio.

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Segregation for number and strength of awns occurred, and the classification of F_2 plants was compared with F_3 breeding behavior. The results could not be explained on any simple genetic basis. There were 66 F_2 plants out of 378 in the cross of II–18–37 × Black Mesdag which were classified in the awnless group. Five of these were selected and their progeny grown in F_3. These bred true for none to few weak awns. The geniculate, strongly awned F_2 types, however, segregated in F_3 for the most part.

Approximately the same number of F_2 plants were selected at random from each of the four groups—black, resistant to rust; black, susceptible to rust; white, resistant; and white, susceptible, and their progeny tested in F_3 to F_5 for smut reaction. Of a total of 378 lines, 86 were classed as susceptible on the basis of F_3 data. These were as susceptible as the II–18–37 parent. Lines which produced no smut in F_3 to F_5 were classed as immune. There were 47 of these lines. Some lines appeared highly resistant and only produced a small percentage of infection. Thirty-six lines were placed in this class. The remainder of the lines, of which there were 209, produced some smut infection although less than the susceptible parent and more than the lines classed as resistant. The susceptible parent produced a small percentage of infection, although each row tested showed some smut each year. The genetic factors for smut reaction can not be determined with accuracy in these studies. Results of the same general nature received here could be obtained by two factors *I* and *R* for immunity and resistance, respectively, both carried in the Black Mesdag parent, each allelomorphic to factors for susceptibility with the further hypothesis of *I* epistatic to *R*. This however, is suggested only as a possible explanation.

Black versus white glumes appeared to be independently inherited from resistance versus susceptibility to rust.

The two classes for color of glumes in F_2 black and white were compared for segregation of awns and for smut reaction by the double X^2 test of goodness of fit. *P* values of 0.9113 and 0.2226 were obtained, which indicates no linkage between factors for color of glumes and for awn development and smut reaction.

There was a slight tendency in all three crosses for a loose linkage between genetic factors for hairs on rachilla and for glume color, although the linkage, if any, was very loose.

No correlation between rust reaction and awn development or rust reaction and smut reaction could be demonstrated by the X^2 test. *P* values of 0.2718 and 0.2697 were obtained. A worse fit could be expected on the basis of random sampling 27 times out of 100 or 1 out of 4.

The coefficient of contingency was used to determine any possible linkage between hairs on rachilla and awn development, hairs on rachilla and smut reaction, and smut reaction and awn development. All three calculated coefficients were low and hardly significant in the light of their probable errors.

From these studies several homozygous lines have been obtained which are resistant to black stem rust, immune from smut, and which have weak awns and white glumes. These are being compared with Victory and Minota for yielding ability.

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VARIATIONS OF THE COLLOIDAL MATERIAL IN TYPICAL AREAS OF THE LEONARDTOWN SILT LOAM SOIL[1]

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INTRODUCTION

Previous studies in this bureau have shown that the behavior of soil in the laboratory is governed very largely by the kind and quantity of colloidal material present (*4*, *15*)[2]. It has also been pointed out that the character of the colloidal material should be considered in soil classification (*9*), since the colloid is distinctively the weathered product of the soil. Information as to the kinds of colloid present in the various cultivated areas is therefore important; and as a preliminary step toward obtaining such data, a study has been made of the colloidal materials present in various areas that have been mapped as a single type of soil.

Data have already been obtained regarding the composition and properties of the colloids present in samples collected from many types of soil (*18*, *10*). These data, collected to show variations of the colloidal soil material in soils in general, can not safely be taken as showing the kinds of colloidal material that are characteristic of the particular types of soil sampled, since none of the types was represented by more than two samples, and the variability of a soil type with respect to colloidal material is unknown. Obviously, unless the colloidal material is fairly uniform in different samples of a type of soil, the nature of the colloid can not be regarded as one of the characteristics of soils as now classified. A priori there is no reason why the colloidal material should be especially uniform throughout an area occupied by a particular type of soil, since the character of the colloidal material is not one of the criteria used in classifying soils. On the other hand, the nature of the colloidal material plays such an important part in determining nearly all properties of a soil that it might be expected to vary little in areas of soil whose characteristics are so nearly uniform as different areas of a single soil type.

The results reported in this paper indicate the variation that may be expected in colloidal materials isolated from different samples of a fairly well-defined type of soil. The data given concern the chemical composition and properties of the colloidal material present in samples of Leonardtown silt loam collected in scattered areas where this type of soil appears well developed.

REVIEW OF LITERATURE

Apparently no studies have been made dealing specifically with variation of the colloidal soil material within a soil type. However, analyses made by several investigators of the clay or colloidal mate-

[1] Received for publication Nov. 22, 1927; issued May, 1928.

[2] Reference is made by number (italic) to "Literature cited," p. 470.

rial isolated from different soils give some indication of the variation that may be expected.

It has previously been pointed out that, although colloids from different soils may differ widely in composition, samples of different soils within a restricted climatic region often contain very similar colloids (*18*). Analyses reported by Hall and Russell (*12*) indicate that four fertile soil types in southeastern England contain clays of approximately the same composition, and three less fertile types from the same regions contain clays which differ very little. Clay fractions of 10 North Wales soil samples reported by G. W. Robinson (*16*) are less uniform in composition. The difference between the highest and lowest silica content of the 10 clays is about 15 per cent of the average content, and the extreme variations of aluminum and iron oxides are, respectively, 34 and 38 per cent of the averages of these constituents. These 10 samples represent three soil types as defined by Robinson. Different samples of a single type show about half these variations. Bradfield (*6*) found the colloidal materials present in subsoils of 11 different soils in Missouri to be fairly uniform in composition. Differences between the highest and lowest silica and alumina contents were, respectively, 8 and 23 per cent of the average quantities present in the 11 samples. Analyses reported by Robinson and Holmes (*18*) of colloidal materials isolated from 45 soil and subsoil samples taken in different parts of the United States include 10 colloids that are fairly uniform in composition. Differences between the highest and lowest silica or alumina contents are only about 8 per cent of the average for these samples, whereas differences between the extremes of Fe_2O_3 or between extremes of combined water are about 20 per cent of the average. It was pointed out as significant that most of these samples are from Maryland—a comparatively restricted area.

The preceding data indicate that in certain regions different types of soil contain colloids which do not differ widely in their major constituents. One would therefore expect a single type of soil to be quite uniform with respect to the kind of colloidal material present.

DESCRIPTION OF SAMPLES

The type of soil examined for variability of the colloidal material was the Leonardtown silt loam. This is a soil of very pronounced characteristics and is therefore fully as well defined as the average soil. Its characteristics, as described by C. F. Marbut in a memorandum to the author, are as follows:

> In the uncultivated Leonardtown silt loam, the features characteristic of the timbered soils of the eastern United States are well developed. The A horizon is usually about 10 inches thick. The first half inch is colored dark by decomposing forest débris and the remainder of A is pale yellow to almost gray. The structure is single-grained with lamination well developed. The upper part of the B horizon, usually 10 to 15 inches thick, is yellowish with a faint reddish brown shade. The nut structure of the particles is so highly developed that the material falls into pieces when at the optimum water content. The outside of the structure particles is lighter in color than the inside, but there is no gray coating. Although the texture is heavier than that of the A horizon, there is no induration. The lower part of the B horizon, beginning usually at the eighteenth to the twenty-fourth inch, is entirely different from upper B. It is so highly indurated that penetration with a soil auger is difficult, and on drying it becomes as hard as sandstone. It is predominantly brownish gray in color, but the colors are unevenly distributed, some spots being gray. It contains many irregularly shaped pores, the wall of which are lined with a brownish coating. The C horizon

beneath the indurated B_2 layer is somewhat variable in character, ranging from a reddish clay to a gravelly clay. In no case is it indurated or cemented.

This type of soil occupies level or nearly level areas in the coastal plain of eastern Maryland and Virginia.

The purpose of this work was not to determine the average composition of the colloidal material in the whole area of Leonardtown silt loam that has been mapped. This study was restricted to determining variability in the colloidal material of soil samples taken in separated areas of Leonardtown silt loam in which the profile is typically developed. The results thus show the variability found in typical Leonardtown silt loam spots and not in all the areas that have been mapped as Leonardtown silt loam.

Three areas near one another and five widely separated were chosen for sampling; otherwise the samples were taken virtually at random. No attempt was made to obtain samples of exceptional uniformity by preliminary examination and rejection of samples. The only precaution observed in selecting a spot for sampling was to see that the profile had well-developed Leonardtown characteristics. The soil samples were not composites but were taken from single holes. The vertical section of soil included in the actual sample thus had a surface area of only a few square inches.

Data regarding the soil samples from which colloidal material was isolated are given in Table 1.

TABLE 1.—*Situation, topography, and vegetation of localities in which samples were collected; horizon and depth from which samples were taken; and colloidal material in samples, estimated by water adsorption and actually isolated from samples*

Locality No.	Locality in which samples were collected	Topography	Vegetation	Horizon	Depth of sample	Colloidal material in sample estimated by water adsorption	Colloidal material actually isolated from sample
					Inches	*Per cent*	*Per cent*
1	2 miles northwest of Forestville, Prince Georges County, Md.	High, flat	Old grass field cover.	A	0–7	10.5	9.1
	Do			B_1	7–17	25.0	21.0
2	15 feet south of locality No. 1	High, flat	Old grass field cover.	B_1	7–17	26.6	21.3
3	200 yards south of locality No. 1.	do	do	A	0–7	10.9	9.4
4	½ mile north of Meadows, Prince Georges County, Md.; 2 miles south of locality No. 1.	Low, flat	Forest	A	0–12	10.7	7.3
	Do			B_1	12–18	26.1	22.5
	Do			B_2	18–28	11.5	10.3
5	½ mile southwest of Mattawoman, Charles County, Md.; 14 miles south of locality No. 1.	Low, flat	Forest	A	0–7	11.9	8.8
	Do			B_1	7–14	25.4	18.0
6	½ mile west of Middleton, Charles County, Md.; 18 miles south of locality No. 1.	Low, flat	Forest	A	0–7	8.9	7.5
	Do			B_1	7–14	18.0	14.8
7	1½ miles west of Laurel, Prince Georges County, Md.; 19 miles north of locality No. 1.	High, flat	Old pasture-land cover.	A	1–8	12.0	9.7
	Do			B_1	8–18	26.1	23.9
	Do			B_2	19–30	15.0	13.6
8	1¼ miles south of Laurel, Prince Georges County, Md.; 17 miles north of locality No. 1.	High, slightly rolling.	Old grass-field cover.	A	1–8		
	Do			B_1	8–18	25.9	23.1

It will be seen that in some cases colloidal material was isolated from three horizons in the profile, but in most cases the examination was limited to the A and upper B horizons.

METHODS

The colloidal material was isolated from the soil very much as described in a previous publication (*10*), except that the distilled water used in the first dispersion of the soil was recovered and used for subsequent treatments of the sample. The water was maintained at a P_H of about 8 by the addition of a few drops of ammonia when necessary. The colloidal material isolated was obviously a fair sample of that in the soil, since the greater part was isolated in each case. The quantities of colloid isolated and the quantities present in the soil as shown by the water vapor absorbed by the soil over 3.3 per cent sulphuric acid are shown in columns 8 and 7 of Table 1. It has been shown that this estimation of the total quantity of colloid is fairly accurate in the case of most soils (*10*).

Analysis of the colloidal material was made by the fusion method. The procedure in most respects was similar to that employed by the Association of Official Agricultural Chemists (*5*), but determinations of TiO_2, MnO, and P_2O_5 were made in accord with Hillebrand's methods for the analysis of silicate rocks (*13*). Organic matter was determined by combustion in an electric furnace, the CO_2 evolved being calculated to organic matter by the factor 0.471. The water of combination was taken to be the difference between the ignition loss and organic matter.

Hydrogen-ion determinations were made electrometrically with a hydrogen electrode essentially as described by Gillespie (*11*). The hydrogen-ion values of the isolated colloids were not determined, since they were altered by the ammonia used in the process of isolation. Determinations, however, were made of the untreated soil. These may be taken as representing approximately the hydrogen-ion concentrations of the unaltered colloid materials, since the P_H of a soil is primarily that of the colloidal material.

The quantity of water vapor adsorbed by the whole soil over 3.3 per cent (by weight) sulphuric acid was determined for the purpose of estimating the quantity of colloid in the soil. The method used has been described in a previous publication (*10*). Determinations were also made of the water vapor adsorbed by the isolated colloids over 30 per cent acid for the purpose of showing differences in the materials. Previous studies showed that different soil colloids adsorb similar quantities of water vapor over 3.3 per cent acid (*17*), but widely different quantities from the drier atmosphere afforded by 30 per cent acid (*3*).

The quantity of ammonia gas adsorbed by the colloidal material was determined by the method described in a previous publication of this bureau (*10*).

The quantity of barium adsorbed from a normal $BaCl_2$ solution was taken as a measure of total base-exchange capacity of the colloidal material. The determination was conducted as follows: 4 gm. of colloidal material were thoroughly agitated with 100 c. c. of a normal solution of $BaCl_2$ which had been adjusted to P_H 7. After standing overnight the mixture was filtered, and the colloidal material was washed on the filter with successive portions of the $BaCl_2$ solution until the total filtrate amounted to 500 c. c. In order to remove the

excess $BaCl_2$, the colloidal material was then washed with distilled water until the filtrate gave no test for chlorides. The adsorbed barium was extracted from the colloid by washing with approximately 700 c. c. of n/20 HCl. Complete extraction of the barium was determined by testing the final washings for barium. The barium in the filtrate, representing the quantity originally adsorbed, was determined as sulphate.

COMPOSITION AND PROPERTIES OF COLLOIDAL MATERIALS PRESENT IN THE DIFFERENT SOIL SAMPLES

The composition of the colloids isolated from the different samples of soil is given in Table 2. In this table the percentages of most constituents are calculated on the weight of total inorganic material (i. e., weight of oven-dried samples minus organic matter) rather than on the weight of the whole sample. Organic matter varies considerably in different samples, and when this is eliminated the percentages of the inorganic constituents become more constant.

In order that variations in the different constituents may be readily compared, the standard deviation and coefficient of variability are given for each constituent. These two constants are calculated by the usual formulas—standard deviation, $\sigma = \sqrt{\frac{\Sigma d^2}{N}}$, and coefficient of variability, $C = \frac{\sigma}{M} \times 100$. In these formulas, d represents the deviation of each determination from the mean, M, of the whole number of determinations, N (*7*).

Apparently, variation in composition of the colloidal material in different localities is shown by samples from either the A or B_1 horizon, since the colloids in these two horizons have very nearly the same chemical composition when the results are calculated on an inorganic basis. Only organic matter and constituents associated with it, such as ignition loss and possibly SO_3 and P_2O_5, differ appreciably in the colloids from these two horizons. The colloidal material in the B_2, or hardpan horizon, also seems similar to that of the A and B_1 horizons. This is certainly true of the profile taken in locality No. 7. In view of the similarity of the colloidal materials in the A and B horizons, any variation found in the colloids of different localities is evidently not due to failure to distinguish sharply between the A and B horizons in sampling the various soil profiles.

Colloidal materials of Leonardtown silt loam from the different localities show considerable variation in organic matter and in those constituents which are present in small quantity—less than approximately one-half of 1 per cent.[3] In organic matter, SO_3 and P_2O_5, the colloids show almost as wide variation as the colloids which were isolated from many different soils in a previous investigation (*18*); hence these constituents do little toward distinguishing the colloids of Table 2 as coming from a particular kind of soil or group of soils. Manganese and lime are also quite variable, but the percentages of these constituents should not be regarded as wholly uncharacteristic

[3] The exact degree of variation in CaO, Na_2O, P_2O_5, and in SO_3 is somewhat uncertain, since in many cases the analytical error is appreciable as compared with the quantities present. But even after a liberal allowance is made for analytical error, it is evident that the samples as a whole are quite variable in those constituents which are minor in quantity. Practically all the differences shown in MnO are real, since this determination is accurate within about two-thousandths of 1 per cent.

TABLE 2.—*Analyses of colloidal materials isolated from samples of Leonardtown silt loam*

Sample from—		Constituents expressed as percentage of the inorganic material (oven-dried colloid minus organic matter)												Constituents expressed as percentage of whole oven-dried colloid including organic matter		Molecular ratio $\frac{SiO_2}{Al_2O_3+Fe_2O_3}$
Locality No.	Horizon	SiO_2	TiO_2	Fe_2O_3	Al_2O_3	MnO	CaO	MgO	K_2O	Na_2O	P_2O_5	SO_3	Combined H_2O	Ignition loss	Organic matter	
1	A	42.56	1.04	12.60	27.46	0.07	0.57	1.06	1.46	0.40	0.46	0.80	11.95	23.54	13.16	2.03
3	A	43.58	.86	10.80	28.50	.03	.22	.76	1.39	.29	.10	.90	12.82	20.27	8.44	2.08
4	A	40.44	.73	10.92	31.01	.03	.23	.88	1.50	.43	.21	.46	12.82	20.28	8.55	1.80
5	A	43.20	.87	12.22	27.70	.08	.47	.89	1.34	.43	.26	.33	11.98	16.30	4.92	2.05
6	A	43.22	.95	12.55	28.51	.05	.45	.78	1.35	.54	.22	.16	11.70	17.10	6.09	2.03
7	A	45.25	.92	11.06	28.18	.08	.33	.76	1.42	.12	.47	.44	11.29	16.81	6.23	2.15
8	A	43.80	.94	12.60	28.54	.08	.43	.97	1.51	.24	.51	.44	10.83	16.22	6.24	2.02
Mean		43.15	.90	11.82	28.56	.06	.39	.87	1.42	.35	.32	.50	11.91	18.65	7.66	2.02
Standard deviation		1.35	.09	.79	1.07	.02	.12	.11	.06	.13	.15	.24	.68	2.57	2.49	.10
Coefficient of variability		3.1	10.0	6.7	3.7	33.3	30.8	12.6	4.2	37.1	46.9	48.0	5.7	13.8	32.5	5.0
1	B^1	42.84	.84	13.56	27.83	.04	.40	1.06	1.31	.36	.19	.50	11.49	15.74	4.80	1.99
2	B^1	42.84	.95	13.95	27.53	.06	.37	.88	1.30	.30	.20	.33	11.36	14.74	3.82	1.98
4	B^1	43.67	.81	12.36	27.73	.03	.21	.88	1.32	.24	.09	.30	11.58	13.67	2.37	2.07
5	B^1	43.25	.86	13.25	27.30	.02	.36	.68	1.05	.37	.15	.18	12.50	14.01	1.70	2.05
6	B^1	42.65	.79	13.59	27.85	.05	.24	.76	1.04	.52	.24	.14	12.98	15.58	2.98	1.98
7	B^1	43.75	.79	14.35	27.00	.02	.14	.92	1.42	.26	.18	.19	10.86	12.68	1.91	2.05
8	B^1	43.00	.86	14.15	28.15	.07	.40	.84	1.43	.23	.24	.18	10.83	12.89	2.31	1.97
Mean		43.14	.84	13.60	27.63	.04	.30	.86	1.27	.33	.18	.26	11.66	14.19	2.84	2.01
Standard deviation		.40	.05	.48	.36	.02	.10	.11	.15	.09	.05	.12	.75	1.3	1.03	.04
Coefficient of variability		.9	6.0	3.5	1.3	40.0	33.3	12.8	11.8	27.3	27.8	46.2	6.4	9.2	36.3	2.0
4	B_2	46.68	.95	7.75	32.34	.01	.26	.50	.68	.20	.17	-------	11.71	12.88	1.33	2.13
7	B_2	44.15	.92	12.97	29.09	.01	.51	1.08	1.30	.20	.12	-------	10.65	12.27	1.82	2.07

of the Leonardtown soil, since the Leonardtown colloids are uniformly lower in these constitutents than many other soil colloids that have been analyzed.

In SiO_2, Al_2O_3, Fe_2O_3, TiO_2, K_2O, MgO, and combined water, the colloids from the different localities vary little. The coefficients of variability range from 0.9 for SiO_2 to 12.8 for MgO. The most constant constituents are SiO_2, Al_2O_3, Fe_2O_3, and combined water, the standard deviations being approximately 5 per cent of the means, as shown by the coefficients of variability. These four constituents make up about 95 per cent of the inorganic material and the TiO_2, K_2O, and MgO account for an additional 3 per cent. Evidently, then, so far as the bulk of the inorganic material is concerned, the different samples of colloidal material are fairly uniform in composition.

In order to characterize further the different samples of colloidal material, some of the more important properties were determined. These included the water vapor adsorbed over 30 per cent sulphuric acid, the quantity of ammonia gas adsorbed at a pressure of 1 atmosphere, the base-exchange capacity as measured by the barium adsorbed from a normal $BaCl_2$ solution, and the hydrogen-ion concentration. Previous studies show that different colloidal soil materials may vary widely in these properties (*10*, *3*). The procedure followed in these determinations is described on page 462.

It should be pointed out that determinations of these properties are probably less reliable measures of variability in the colloid samples than the chemical composition, since it is quite possible that some of the properties of the colloids were altered by the procedure of isolation. Previous studies show that in the isolation of certain colloids the heat of wetting and the adsorptive capacity are altered appreciably (*1*, *10*). Doubtless, in the case of these samples there was some alteration in the capacities for adsorbing water, ammonia, and barium; but since the colloidal materials were all very much alike and were isolated by the same procedure, it is to be presumed that they were all altered to approximately the same extent. Also, too much importance should not be attached to the P_H determination as an indication of variability, since this determination was made on the whole soil, and it may have been affected somewhat by the noncolloidal constituents of the soil. The results of this group of determinations are given in Table 3.

It is apparent from Table 3 that the colloids from the different localities are fairly constant in four important properties. In hydrogen-ion concentration and in adsorptive capacity for water vapor and ammonia, the variability is about the same as that shown by the four major chemical constituents (SiO_2, Al_2O_3, Fe_2O_3, and combined water). The variability in base-exchange capacity (Ba adsorbed) is somewhat greater; it is about the same as the variability in K_2O, MgO, and TiO_2.

Thus far, variability of the colloid samples has been judged from the variation in separate constituents and single properties. Obviously, this variability of single constituents does not show how constant the samples are in their whole composition, since one sample may be decidedly different from the other samples in one or two constituents but quite similar in all others. A better idea of how constant the samples are, taking everything into consideration, may

be gained from Table 4. In this table the quantities of the more constant chemical constituents and the values for four properties are expressed relative to the means of the A and B_1 colloids.

TABLE 3.—*Properties of the different samples of colloidal material*

Samples from—		Quantity of water vapor adsorbed per 100 gm. of colloid	Quantity of NH_3 gas adsorbed per 100 gm. of colloid	Quantity of Ba adsorbed per 100 gm. of colloid	Hydrogen-ion concentration of untreated soil
Locality No.	Horizon				
		Gm.	*Gm.*	*Milli-equivalents*	P_H
4	A	5.69	2.69	2.30	4.6
5	A	5.50	2.45	2.10	4.6
6	A	5.11	2.52	1.85	4.6
7	A	5.37	2.56	1.51	4.5
8	A	5.73	2.85	2.11	4.5
Mean		5.48	2.61	1.98	4.6
Standard deviation		.21	.14	.27	.02
Coefficient of variability		3.8	5.4	13.6	.4
1	B_1	7.06	2.65	2.60	4.9
2	B_1	7.41	2.86	2.55	4.6
4	B_1	7.43	2.97	2.33	4.5
5	B_1	6.68	2.56	2.15	4.9
6	B_1	6.33	2.67	2.15	4.7
7	B_1	6.61	2.76	2.38	5.0
8	B_1	7.24	2.91	2.33	4.9
Mean		6.97	2.77	2.36	4.8
Standard deviation		.40	.14	.16	.17
Coefficient of variability		5.7	5.1	6.8	3.5
4	B_2	5.54	2.64	2.55	5.1
7	B_2	9.29	2.85	3.41	4.7

TABLE 4.—*Composition and adsorptive capacity of the various samples, expressed relative to the means as 100*

Sample from—		The more constant chemical constituents of the colloid								Adsorption capacity of the colloid for—			P_H
Locality number	Horizon	SiO_2	TiO_2	Fe_2O_3	Al_2O_3	MgO	K_2O	Combined H_2O	Mols $\frac{SiO_2}{Al_2O_3+Fe_2O_3}$	Water vapor	Ammonia gas	Ba from $BaCl_2$ solution	
1	A	99	116	107	96	122	103	100	100				
3	A	101	96	91	100	87	98	108	103				
4	A	94	81	92	109	101	106	108	89	104	103	112	100
5	A	100	97	103	97	102	94	101	101	100	94	107	100
6	A	100	106	106	100	90	95	98	100	93	97	94	100
7	A	105	102	97	99	87	100	95	106	98	98	77	98
8	A	101	104	107	100	111	107	91	100	105	109	107	98
1	B_1	99	100	100	101	123	103	99	99	101	96	110	102
2	B_1	99	113	103	100	102	102	97	98	106	103	108	96
4	B_1	101	96	91	100	102	104	99	103	107	107	98	94
5	B_1	100	102	97	99	79	83	107	102	96	92	98	102
6	B_1	99	94	100	101	88	82	111	99	91	96	91	98
7	B_1	101	94	105	98	107	112	93	102	95	100	100	104
8	B_1	100	102	104	102	98	113	93	98	104	105	98	102

When the seven constituents and four properties shown in Table 4 are considered together, it appears that the different samples of colloidal material are quite uniform in character. If a variation of 10 per cent from the mean is taken as "appreciable," it will be seen that only one of the colloids, 6–B_1, differs appreciably from the mean in 3 of the 11 characteristics tabulated. Most of the colloids vary

appreciably in only 1 constituent or property. All appreciable variations, except one, occur in the TiO_2, MgO, or K_2O, which together form only about 3 per cent of the whole colloid. Apparently, colloids taken from widely separated localities agree with one another as closely as colloids taken from practically adjacent spots. Samples 6 and 7, for instance, agree with each other or with samples 1, 2, and 3 about as closely as sample 1, 2, and 3 agree with one another; yet Table 1 shows that the localities from which samples 6 and 7 were taken are about 37 miles apart, whereas those of samples 1, 2, and 3 are only 5 and 200 yards apart.

In view of these data, it seems justifiable to conclude that the Leonardtown silt loam soil is characterized by a fairly definite kind of colloidal material. The typical Leonardtown colloid appears to be defined by its P_H, by its absorptive capacity for water, ammonia, and barium, and by its content of SiO_2, TiO_2, Al_2O_3, Fe_2O_3, MgO, K_2O, and combined water. The percentages of organic matter, SO_3, and P_2O_5 are too variable to be characteristic, and the contents of lime and manganese are definitive only in being low.

In connection with the conclusion that a type of colloid is characteristic of the Leonardtown type of soil, the nature of the soil sampling should be considered. As previously pointed out, the soil samples used in this work were not composites but were taken from single holes and embraced only a few cubic inches of soil. Since these small samples yielded uniform colloids, it is evident that with respect to colloidal material the Leonardtown type of soil is a fairly definite object even in small units.

Some idea of the number of samples that should be taken in order to determine the type of colloid that is characteristic of a soil type may be gained from the data given in Table 2. By use of the usual formulas for probable error,[4] the accuracy of the mean compositions given in the table and the limits within which a single sample should approach the mean may be estimated. It appears that in the case of the A-horizon colloids the chances are even that the means given in Table 2 agree with the true means within ± 0.34 per cent SiO_2, ± 0.20 per cent Fe_2O_3, ± 0.28 per cent Al_2O_3, and ± 0.17 per cent combined water, and there is an even chance that a single sample would agree with the mean within ± 0.91 per cent SiO_2, ± 0.53 per cent Fe_2O_3, ± 0.72 per cent Al_2O_3, and ± 0.46 per cent combined water. In the case of the B_1-horizon colloids the probable errors of sampling are somewhat less, since these colloids are slightly more constant than the A-horizon colloids. Evidently, from 8 to 10 samples of a soil type would be sufficient to show the kind of colloid characteristic of the soil with a fair degree of surety.

Incidentally, the data obtained in this study are important in showing how closely different properties of the colloidal soil material may be expected to correlate with one another and with the chemical composition of the material. In previous studies of widely different colloidal soil materials, fair correlations were found between variations of the colloids in one property and variations in another; correlations were also apparent between variations in properties and

[4] The probable error of a single determination $=0.6745\sigma$ and the probable error of the mean $=\frac{0.6745\sigma}{\sqrt{N}}$. In these formulas σ represents the standard deviation and N the number of determinations.

variations in the $\frac{SiO_2}{Al_2O_3+Fe_2O_3}$ ratio (*2, 8, 3*). These relationships were established for a series of colloids in which wide variation occurred. In this series of colloids the variations are small; hence the relationships would probably appear less marked, the relationship not being absolute. It appears from Table 4, however, that in the case of these very similar samples of Leonardtown colloid there is some correlation between properties. The figures given in Table 3 for the adsorption of water vapor and ammonia gas show a fair correlation, the correlation coefficient between these two determinations being 0.71 ± 0.15 for the A-horizon colloids and 0.75 ± 0.11 for the B_1-horizon colloids. The quantities of barium and water vapor adsorbed are less closely correlated, the coefficients for the A and B_1 colloids being 0.66 ± 0.17 and 0.58 ± 0.17, respectively. There is no significant correlation between the adsorptions of barium and ammonia, and the adsorption data do not correlate with the comparatively constant $\frac{SiO_2}{Al_2O_3+Fe_2O_3}$ ratio. The slight correlation of properties in the case of these colloids of low variability is in harmony with the results of previous studies. It also serves to emphasize the fact that correlations between different properties and correlations between properties and chemical composition of the colloidal soil material are far from perfect.

DISCUSSION

The preceding data show that fairly typical spots of Leonardtown silt loam, selected at random, contain colloidal materials which are quite constant in their major constituents and adsorptive capacities. The nature of the colloidal material may therefore be considered a characteristic of this soil type. Further investigation is needed to show how constant other types of soil are in colloidal material; but it is fairly certain that the nature of the colloid may be considered a characteristic of many, if not all, types of soil.

Even if further investigation shows that each soil type is constant in its colloidal material, many data must be collected before it is evident just how valuable the composition of the colloid is as a classification characteristic. On the basis of the four major constituents $\frac{SiO_2}{Al_2O_3+Fe_2O_3}$ or of the ratio, only a few groups of soil types could be distinguished; but some of the more variable minor constituents will doubtless distinguish additional subordinate groups. It is not unreasonable to expect that the kind of colloidal material will prove as valuable a characteristic for soil classification as the quantity of clay or colloid has proved to be.

Aside from any value in soil classification, a knowledge of the kinds of colloidal material in the different cultivated areas is important for throwing light on the properties and management of soils; and it appears from this study that such knowledge may be approximated by analyzing the collodial materials present in a comparatively few samples typical of each type of soil. The probable error calculations given on page 467 show that in the case of the Leonardtown silt loam, seven samples of soil taken with only reasonable care showed

the kind of colloid typical of this soil type with considerable accuracy, and that samples of colloid from individual spots do not show a wide variability in their major constituents. Of course, it does not follow from these data that all areas that have been mapped as Leonardtown silt loam will contain colloidal materials as similar to the mean as the typical samples that have been analyzed. But the colloid of any particular area should at least be similar to the mean of the type in proportion as it is typical Leonardtown, since typical spots are fairly constant.

The data given in Table 2 throw light on two questions that are not immediately related to this study. It was pointed out that the colloidal material from the A horizon has very nearly the same composition as that of the B_1 horizon when the results are expressed on an inorganic basis. This is in agreement with the results of a previous investigation in the course of which analyses were made of colloids isolated from the A and B horizons of 15 types of soil (*18*). In each case the A horizon colloid was very similar to the B colloid in inorganic composition. It thus seems that the colloids in these two horizons are similar in most types of soil. Results recently obtained by McCool on some Michigan soils indicate, however, that this is not universally true (*14*). The fact that colloidal material in the B_2 horizon is also very similar to that in the A and B_1 horizons is somewhat surprising, since the B_2 horizon is a distinct hardpan. Data given in Table 1 shows that this indurated layer contains considerably less colloidal material than the friable B_1 horizon; hence it would appear that the hardness characteristic of this layer is due neither to the kind nor to a particular quantity of colloidal material present. Possibly it is primarily dependent on a peculiar structure of the noncolloidal material.

SUMMARY

This study was conducted for the purpose of determining the variability in the colloidal material of typical samples of Leonardtown silt loam soil. The data reported concern the chemical composition and properties of the colloidal materials that were isolated from random samples typical of this silt loam.

The colloids of this type obtained from different localities are, on the whole, fairly constant in hydrogen-ion concentration, in adsorptive capacity for water vapor, ammonia gas, and barium, and in content of SiO_2, Al_2O_3, Fe_2O_3, combined water, TiO_2, K_2O, and MgO. The MnO and CaO are more variable, but these constituents are low in all samples. Organic matter, SO_3, and P_2O_5 vary widely. It thus appears that the Leonardtown silt loam type of soil is characterized by a type of colloid which is fairly definite in adsorptive properties and in those inorganic constituents that are present in appreciable quantities.

The probable errors of sampling indicate that 8 to 10 samples of a type of soil should be sufficient to show with considerable accuracy the kind of colloidal material characteristic of the type.

Incidentally, the data obtained in this study confirm a conclusion of previous work, that in most soils the colloidal materials of the A and B_1 horizons are very similar in inorganic composition. The data also indicate that the hardness of the B_2 horizon in this type of soil is not primarily dependent upon the kind or quantity of colloidal material present.

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A CHEMICAL STUDY OF THE DEVELOPMENT OF COTTON BOLLS AND THE RATE OF FORMATION OF GOSSYPOL IN THE COTTON SEED.[1]

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INTRODUCTION

The significance of small amounts of gossypol and its related compounds in cottonseed products has been emphasized in previous communications (*6*, *7*).[3] In a series of investigations to determine the factors which affect the gossypol content of cottonseed meal it became necessary to study the rate of formation of gossypol in the cotton seed and to determine the stage of development of the seed at which gossypol was present in the greatest amounts. The quantity of this toxic substance in the meal is largely dependent upon the quantity in the original seeds, and this in turn is probably governed somewhat by the nutrition of the plant. Evidence of the existence of such a dependent relationship has been previously reported (*15*), and a positive correlation found to exist between the gossypol content of the seed and its oil content. The practical application of such findings, however important, has not received a great deal of attention.

In a previous investigation (*7*) it was observed that gossypol began to form in the cotton seed previous to the opening of the boll and reached its maximum amount soon after the boll had opened. The oil developed in the seed in increasing amounts at the same time, although not in the same proportion. The age of the seeds was calculated from the stage of development of the boll.

EXPERIMENTAL

In the study reported here, which had for its purpose the further investigation of the apparent relationship existing between the formation of gossypol and of oil during the development of the seed, more rigid measures were taken to determine the exact age of the experimental material and reduce variability in samples to a minimum. To reach this objective and make the results comparable to those obtained a year previous and recently reported (*7*), the same variety of cotton seeds was used and the seeds were obtained from plants growing in the same area of the field. When the plants were in bloom the flowers were marked with the date at which they were fully open, and this date was used to determine the age of the bolls which were later collected. A sufficient number of flowers were

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[3] Reference is made by number (italic) to "Literature cited," p. 480.

marked during 10 days of the flowering period to insure a large quantity of representative samples. Eight days after the first flowers were marked a collection of bolls was made and further collections were made at the intervals shown in the tables which follow. By this procedure a considerable amount of experimental material other than the seeds which were needed for the gossypol determinations was collected, and it appeared worth while to include in the study the analysis of the several parts of these cotton bolls as they matured.

Although an enormous amount of work has been done on the chemical composition of the cotton plant as a whole, little attention has been given to the composition of the contents of the boll in the various stages of development, and it seemed for several reasons that this might prove of some importance. The need for specific information of this sort is felt most in finding new uses for waste products which accumulate each year. In fact, the data collected during this part of the investigation yielded such interesting results that they tended to subordinate the primary object of the study, which was mainly concerned with the formation of gossypol and oil in the cotton seed.

Unopen or partly open bolls which fail to mature and are left in the field have practically no value at the present time, although in some years it has been a practice at the close of the cotton-picking season to collect these bolls and sell them to the ginners. Bolly refuse, consisting mainly of the burr and some unginned cotton and which collects at the cotton gins in large quantities, has been suggested as a feed of some value (*5*). The ash of this material is high in fertilizing constituents. From the results presented in this paper the composition of cotton lint itself might be expected to show some variation from year to year, a fact which would impair its value for certain specialized purposes. Brown (*3*) quite correctly states that "cotton lint is often spoken of as being pure cellulose, but * * * it contains several other materials in considerable quantity."

For the sake of brevity and to facilitate the comparison of a large number of analyses, the results of this study are presented in several tables with a discussion following each. The proximate analyses were made by the methods of the Association of Official Agricultural Chemists (*1*) and the gossypol determined by Carruth's method as modified by Schwartze and Alsberg (*15*).

DISTRIBUTION OF MATERIAL IN COTTON BOLLS

The weight of the dry material obtained from 25 bolls of cotton at different stages of maturity is shown in Table 1. In the data here presented a small increment of error may have been introduced through the use of the calculated, rather than the actual, weight of some of the materials. It is unfortunate for the completeness of this table that the cotton burrs were not preserved during the first part of the experiment, but as their analyses would be of little value to the main object of the study, the calculation of these values seemed justifiable.

TABLE 1.—*Weight of dry material obtained from 25 bolls of cotton at different stages of maturity*

Age of material	Size of bolls	Weight of—					
		Bolls	Contents of bolls	Lint	Seeds		Burrs
	Cm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*		*Gm.*
8 days	2.5	25.16					
16 days	3.4	84.25	46.86	4.83	30.35	[a] (870)	[b] 37.39
24 days	3.5	121.65	62.19	13.40	31.52	[a] (877)	[b] 59.46
32 days	3.5	136.84	109.90	41.56	51.28	[a] (725)	[b] 26.94
46 days	3.5	212.73	171.20	56.50	94.85	[a] (862)	41.53
52 days		214.89	169.60	60.75	117.87	[a] (1,047)	45.29

[a] Number of seeds in 25 bolls.
[b] This figure represents the calculated rather than the actual weight.

It may be noticed that the combined weights of the dry seeds and lint of 25 bolls do not agree with the dry weights of the boll contents, and that after 24 days this percentage difference becomes less at each successive stage of maturity. These differences are taken as representing water-soluble material removed from the seeds and lint during the preparation of the samples, as described later. Moreover, the figures given are the averages obtained from a large number of samples calculated on the basis of 25 bolls, and not the weights of material obtained from a few selected samples, which would no doubt give a distorted picture of actual conditions, due to a surprisingly large variation in size of bolls.

The separation of the lint from the seeds was done by hand and complete separation was easily accomplished in those instances in which the material was very wet. As the seeds became more mature the lint was more firmly attached and its final removal was effected with sulphuric acid. By determining the dry weight of a given number of seeds before and after delinting, the residual lint, more properly termed "fuzz" or "linters," was found and the proper correction made for it in determining the total amount of lint in the bolls.

The number of seeds in 25 bolls, which is given in parenthesis to the right of the weight of the seeds, shows some variations that must be taken into account in the analysis given later. These variations, which undoubtedly contribute an element of error when the percentage composition of the material produced by the plant is used as an index of quality without taking into consideration the quantity produced, was controlled as much as possible by discarding the bolls showing insect injury or abnormal conditions. Fortunately, very few bolls at any of the stages given were unsuitable as experimental material. The bolls 32 days old yielded the smallest number of seeds and those 52 days old the largest number, while the others were quite uniform in respect to number present. The mature bolls should contain no more seeds than the immature ones, although this is only true providing the development of some of the seeds has not been prevented by some injury which might have passed unnoticed in the immature bolls but which was sufficiently apparent in the mature boll to mark it as unsuitable for analysis. With these explanatory remarks, and excepting the number of seeds found in the bolls 32 days old, the author believes that Table 1 represents the average distribution of material in healthy cotton bolls from plants of Oklahoma 44 variety grown under normal conditions.

CHEMICAL ANALYSES

The results of the analyses of the cotton bolls and their contents are given in Table 2.

TABLE 2.—*Chemical composition of cotton bolls and contents at different stages of maturity, determined on a dry-matter basis*

Age of material	Ash	Crude protein	Crude fiber	Nitrogen-free extract	Ether extract
		BOLLS			
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*
8 days	4.16	13.91	7.03	70.68	4.22
16 days	4.10	14.24	16.50	62.32	2.84
24 days	3.80	12.44	24.86	56.09	2.81
32 days	3.57	11.81	40.20	37.00	7.42
46 days	3.81	13.72	46.89	22.94	12.64
52 days	3.93	12.75	51.27	21.28	10.77
		BOLL CONTENTS			
16 days	4.28	13.96	12.62	65.92	3.22
24 days	3.36	12.01	27.50	53.52	3.61
32 days	2.98	13.36	46.27	28.20	9.19
46 days	2.55	15.50	47.00	19.81	15.14
52 days	2.50	14.60	53.23	16.65	13.02
		LINT			
16 days	2.85	10.63	46.57	35.11	4.84
24 days	1.03	4.75	72.68	18.80	2.74
32 days	.62	1.67	88.98	8.19	.54
46 days	.34	.23	89.66	9.49	.28
52 days	.42	.42	93.34	5.53	.29
		SEEDS			
16 days	3.15	16.04	8.97	70.84	1.00
24 days	3.75	17.27	19.56	56.30	3.12
32 days	3.76	22.89	19.56	31.44	22.35
46 days	3.48	26.95	18.20	26.25	25.12
52 days	3.53	26.96	19.12	25.42	24.97

BOLLS

In the previous discussion, attention was called to the manner in which the age of the bolls was determined by dating the flowers and counting the number of days from that date until the bolls were picked. The cotton flowers usually wither and fall off the second day after they open and the boll which develops from the enlarged ovary of the flower rapidly grows to full size. After each picking the bolls were brought to the laboratory and weighed. They were then dried at 100° C. for one hour or slightly longer, and finally air dried. Analysis was made immediately on the ground air-dry material and the results computed to the dry-matter basis. The bolls eight days old were so small and undeveloped that a separation of their parts for analysis was not attempted.

As the bolls matured the percentage of ash and crude protein remained quite constant, nitrogen-free extract decreased rapidly, and crude fiber and ether extract both increased. None of the bolls

had opened until after the fortieth day and in the collection made on the forty-sixth day only 10 in 100 had opened. The composition of these unopened bolls was much the same as the composition of mature bolls which were entirely open and picked six days later. Although this similarity in composition is not startling, it is of some consequence and worthy of attention. Dowell and Friedemann (*5*) made analyses of the seeds obtained from bollies (the term applied at the cotton gin to unopen bolls) and compared the properties of the oil obtained from such seed with those of the prime crude oil. They were unable to draw definite conclusions as to the value of the bolly oil and were of the opinion that the composition of the seeds would vary from year to year. From the data they presented one might expect the oil to be of inferior quality. Other investigators have reported the analysis of cotton bolls at different stages of development, but for the most part these analyses have been made on material of which the age was only approximate, or else certain assumptions were made which rendered the results of comparative interest only, although suitable for the purpose of the particular investigation. The earliest of these investigations are reported in Bulletin No. 33, United States Department of Agriculture, Office of Experiment Stations (*16*).

BOLL CONTENTS

The contents from a selected number of bolls were handled as described above and care was exercised to retain in the boll contents all the material which belonged to them. In this material the percentage of ash showed a decrease as the boll matured and as the lint, which is low in ash, developed. The protein remained quite constant since nitrogen is present in relatively large amounts at an early stage and increases in the seeds as it decreases in the lint during the period of maturation. Both crude fiber and ether extract increased rapidly. Further comment on these results is not necessary.

LINT

The composition of cotton lint, which is of little importance from the standpoint of the demands made upon the fertilizing constituents of the soil during the growth of the plant, is of considerable importance in relation to the utilization of the product. As previously pointed out, and contrary to the opinion of many not directly connected with the problems involved in textile work, the lint contains appreciable amounts of material other than cellulose. The presence of these materials, sometimes referred to as natural impurities, were recognized as early as the middle of the ninteenth century, although it was not until comparatively recent years that any great significance was attached to them. The only other study made upon the composition of cotton lint during successive stages of development, with which the writer is familiar, is one reported by Levine (*10*) several years ago. The study mentioned was carried out to elucidate results of a previous investigation by Hebden (*9*), who was probably the first to point out the possibility that the proteins of the fiber play an important part in the bleaching of cotton cloth. Levine determined the protein content and the content of ether and alcohol-

soluble material in the fibers at two-day intervals from the time the boll was about 16 days old until it was 38 days old. Although this investigator did not specify the manner in which the experimental material was handled nor the basis on which his calculations were made, the results were of such importance that he pointed out the necessity of careful investigation of the nature of the fatty and waxy substances and a study of the effect of growth on these constituents of the cotton fiber. It is in connection with this latter aspect of the problem, as well as the protein content of the fibers, that certain studies have been promoted at this station.

TABLE 3.—*Chemical composition of cotton lint as reported by different investigators*

Approximate age of lint	Ash	Crude protein	Crude fiber	Nitrogen-free extract	Ether extract	Analyst
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	
16 days		13.94				Levine.
26 days		2.35			1.74	Do.
32 days		1.66			1.52	Do.
38 days		1.13			1.41	Do.
52 days	0.42	.42	93.34	5.53	.29	Gallup.
	1.77	1.61	89.75	6.22	.65	McBryde (*11*).
	1.25	1.12	87.02	10.00	.61	Ross (*14*).

In Table 3 are reproduced the results of several analyses of cotton lint reported by different investigators. These figures are themselves sufficient evidence for assuming the composition of the lint to be variable and worthy of further study. The figures presented in Table 2, which represent the composition of the lint at different stages of maturity, were obtained by analyzing samples washed free of adhering material. When the bolls were collected, the lint was immediately separated from the seeds and washed several times with cold water. Each sample was treated alike, and although such a procedure is subject to some criticism, it no doubt affords the best means of obtaining representative samples, which would be impossible if the total contents of the boll without the seeds were taken as representing lint.

SEEDS

Many analyses of mature cotton seeds have been made by various investigators, but little attention has been given to the rate of formation and development of the oil in the seed. The manufacturer of cottonseed oil is primarily interested in the amount of oil in the seed, and, although its protein content is of secondary importance, in late years there has been a growing endeavor on the part of the purchaser to obtain seeds high in both. This increasing demand for seeds of high oil content has prompted plant breeders and others to attempt to increase the oil and protein content of seeds by proper selection and various fertilizer treatments. The work of Garner, Allard, and Foubert (*8*), Wells and Smith (*17*), Creswell and Bidwell (*4*), and in particular Rast (*13*)—who concludes that "by eliminating the inferior varieties the quality of the seed could easily be improved, thereby increasing their average value $5 per ton"—

has gone far toward promoting studies along this line. Many others have contributed information of equal importance.

An inspection of the figures in Table 2, showing the rate of formation of oil in the seeds, leads one to believe that there is a period of intense oil formation occuring in the seeds 32 days old. This might at first appear to be at variance with what has been previously reported and is reproduced with the later results in Table 4. In the year 1926, when a similar study was made, the exact age of the seeds analyzed was not known, and since the size of the boll would be of no value in determining this, its condition with respect to opening was taken as the most logical basis for determining the stage of development of the seed. For that reason the results do not lend themselves to direct comparison with those obtained in this later study unless certain reservations are made. Again referring to the figures in Table 2, with special reference to the changes in oil and protein, it may be pointed out that the increase in protein as the seeds develop is much less than that in oil and shows no critical period of formation. The increase of both is relatively slight during the last 20 days. From this it might be reasoned that there is a deposition of nitrogen early in the life of the seed, and that its increase, though fairly constant, is proportionally greater than that of the growth of the seed. In contrast to this, the oil developed at a well-defined stage of maturity without showing any relation to the growth of the seed before or after this critical period. Certainly some one or more factors other than the actual age of the seed must have been responsible for the low percentage of oil recovered by Dowell and Friedemann (*5*) in their studies with bolly refuse and by Garner, Allard, and Foubert (*8*) in their work with seeds from immature and mature bolls. Perhaps these differences may be explained on the assumption that the composition of the seed is influenced by seasonal variations, and for this there is some experimental evidence. Martin, Ballard, and Simpson (*12*) found that the period of maturation of the seeds increased as the season advanced. In another investigation (*8*) it was concluded that climate was a potent factor in determining conditions favorable to formation of oil. Other studies (*2*) have shown a close relation between the amount of rainfall during the cotton-growing season and the amount of oil in the seeds. Since the bolls are formed on the plants at different times, the earliest ones being found toward the bottom of the plant and close to the main stem, it is quite obvious that the period of oil formation occurring in the late-developing seeds may be subject to vastly different and often unfavorable growing conditions. A systematic study of the development of oil, or any other constitutent in the seed, with the hope of increasing the amount of any one of them would therefore require special precautions whereby all variables could be controlled and kept fairly constant. These points, which should be borne in mind when interpreting the experimental data presented in Table 2, have special significance in attempting a comparison of results such as has been made in Table 4.

TABLE 4.—*Development of oil and gossypol in cotton seeds*

Description of material	Oil in seeds	Gossypol in seeds
AS PREVIOUSLY REPORTED		
	Per cent	*Per cent*
Unopen bolls	13.97	0.48
Open bolls	24.01	.428
Bolls open over six days	24.41	.538
PRESENT INVESTIGATION		
Bolls 16 days old	1.00	
Bolls 24 days old	3.12	
Bolls 32 days old	22.35	0.230
Bolls 46 days old	25.12	.634
Bolls 52 days old	24.97	.538
AVERAGES REPORTED BY OTHER INVESTIGATORS		
Bollies [a]	12.20	[b]
Immature bolls [c]	20.4	[b]
Mature bolls	23.5	[b]

[a] Dowell and Friedemann (*5*). With reference to the oil content of the seeds from bollies, the investigators state that no definite conclusions can be drawn as the composition of the seeds will probably vary from year to year. No doubt these seeds, which were obtained from various oil mills, represented a more heterogeneous sample than any of the other ones represented.

[b] Not determined.

[c] Garner, Allard, and Foubert (*8*). These investigators state that "immature samples were taken when the green bolls had reached full size and had begun to show numerous brown spots." From this it is presumed that the immature bolls were about 40 days old.

FORMATION OF GOSSYPOL

The seeds on which the gossypol determinations were made after removing the lint were dried at a sufficiently low temperature to prevent the destruction of gossypol. Otherwise they were handled in the same manner as the other materials. From the figures presented in Table 4, it is quite evident that the formation of gossypol was concurrent with that of oil, and from these figures the idea might be gained that some relation existed between the two. In view of the importance of such a relation, in considering the function of gossypol, the experimental basis for such a view calls for careful consideration and far more data than have been obtained up to the present time. Too little is known about gossypol to even postulate that it might act as an accelerator for the formation of oil, although such a conception is no more far-reaching than many others which have been made and later proved. Gossypol could not be detected in seeds from bolls less than 32 days old and no doubt the ether-extracted material of the seeds previous to this time was made up in part of soluble material other than oil. However, there still remains the possibility of gossypol being present in these younger seeds in a form which is not ether soluble and does not respond to the usual tests for gossypol.

It was observed during the extraction with ether of the fat-soluble material from the seeds that the color of the first portion of the extract which ran through was red only in the case of the more mature seeds. The seeds from bolls 16 and 24 days old gave no such color to the extract and contained very little oil. Although this red color is characteristic of solutions of gossypol, other mate-

rials of a questionable nature and present largely in the seed coating give a similar color, and it is probable that all of these made their appearance at about the same time. The absence of red very likely indicates the absence of even small traces of gossypol, but this should not be taken as conclusive proof.

It should be kept in mind that the age of the seeds whose oil and gossypol content are shown in the first part of Table 4, was approximated by observing the condition of the boll; a procedure which, as previously indicated, may not be entirely reliable. It would be hardly possible to discriminate by appearance alone between bolls 24 and 32 days old, and even when the first signs of boll opening are taken as representing material of about the same age, one is confronted with the possibility of short maturation periods with subsequent variations in the final composition of the seeds. Such an explanation may account for the small amount of gossypol and relatively large amount of oil found in the seeds from bolls designated as unopen. What has been said in this respect will also apply to the oil content of seeds from bollies.

Schwartze and Alsberg (*15*) made the important observation that the variation in the gossypol content of seeds tended to vary directly with and bore a true relationship to the oil content. Seeds from the Southwest tended to be low in oil and gossypol, those from the Southeast to be somewhat higher, and those from the Pacific coast regions to be still higher. This again points to the influence of climatic conditions on the development of the oil and the above relationship is unfavorable from the viewpoint of producing seeds high in oil and low in gossypol. If the latter situation could be met it would be of value only in so far as it could be accomplished without detriment to the other ingredients in the seed. Such an achievement would indeed be a valuable contribution to the cottonseed oil industry and all others concerned with its products.

SUMMARY

A study of the change in composition of cotton bolls picked at different stages of development showed that the changes were very rapid from the time the boll first formed until it was ready to open. The young bolls were high in ash, nitrogen, and carbohydrates, and as they matured the fat and crude fiber increased. After the bolls had reached the opening stage their composition was altered slightly by an increase in crude fiber, but from the results presented, the nearly mature but unopen bolls will yield products almost as high in their several constituents as the open ones. The composition of bollies and bolly refuse which are composed of immature bolls and their parts in various stages of development, seems to depend upon a number of factors, the most important of which is probably the age of the boll, followed by time of maturation, climatic conditions during growth, and possibly the nutrition of the plant.

Cotton lint is quite variable in composition and although the mature lint is composed mostly of crude fiber, there are other materials present in sufficiently large amounts to be of questionable importance.

The development of oil and gossypol in the cotton seed occurred at about the same time and during a very short and well-defined period

of growth. Although it is too early to predict the function of gossypol in the development of the seed, it seems to be associated in some way with the formation of oil. A careful study of all the factors which may influence the growth of the seed, together with an endeavor to increase the oil and decrease the gossypol, is needed.

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AN OBSERVED CASE OF "SPONTANEOUS" IGNITION IN STABLE MANURE.[1]

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Instances of "spontaneous" ignition of farm products which have occurred under actual observation are sufficiently rare to warrant the publication of all such available data. Studies of the agents active in "spontaneous" heat production have been conducted by the senior author for several years, but it was only by good fortune that the present writers were enabled to study a case of fire at first hand.

A number of authentic detailed accounts of "spontaneous" ignition in farm products have been reported in the past decade.[2] That fires in such materials do occur from heat generated within the mass can be doubted no longer, though some observers still attribute the conflagration to sparks, carelessly discarded matches, etc.

The observations reported in this paper were made upon a large pile of stable manure and straw at the Arlington Experiment Farm, Rosslyn, Va. Manure for fertilizer purposes had been hauled from a neighboring cavalry station and placed on the farm grounds in an open plot. As the manure was unloaded the horses and wagons were driven over the pile, and the load was deposited on the top. The custom had been to "cure" the manure from one to three years before spreading it. The first loads had been deposited some two and one-half years before, and the mass had grown to a pile about 200 feet long, 50 feet wide, and from 1 to 20 feet high. (Fig. 1.) Moderate heating of the manure had been accepted as an essential part of the curing, though fire had not formerly occurred.

Before September 9, 1925, the date of the outbreak, the weather had been warm, ranging from 87° to 95° F. There had been no heavy rains for two weeks. Daily additions had been made to the pile, and, although the mass was known to be excessively hot, the condition was not considered dangerous. Fire broke out during the night of September 9, and was discovered by the night watchman. The flames quickly spread over the entire west side of the long pile. Water was applied from pails, and the flames were extinguished. Within a few hours, fire again broke out along the same side of the mass. When this was controlled, efforts were made to cut away as much of the material as was still at a dangerously high temperature. Charred straw and manure to a depth of about 2 feet throughout the greater part of the length of the pile were removed. The material was firmly packed, black, dry, and brittle to a depth considerably beyond any possible penetration by the fire. (Fig. 2.)

[1] Received for publication Dec. 24, 1927; issued May, 1928.

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The temperatures of various points in the mass were determined with the aid of a thermocouple and potentiometer. Holes were made by forcing a long iron rod into a pile; the thermocouple was

FIG. 1.—Pile of stable manure, showing area of third outbreak of fire; east side

inserted in the openings. A series of readings (Table 1) indicated that in the center of the pile and up to within 5 or 6 feet of the outer surface the temperatures were not extremely high, averaging only

FIG. 2.—Site of first and second outbreaks of fire, showing compact character of manure pile; west side

about 51° C.; 66° was the maximum. The temperatures of the outer layers, however, were considerably higher. In places where the manure was firmly packed, as on the dismantled side, the highest temperature was usually within 6 inches of the surface, whereas on the top, where recent additions still lay more or less loosely, the maximum temperature was usually found from 1½ to 2 feet within the mass.

TABLE 1.—*Temperatures obtained with a thermocouple in different areas of a large pile of stable manure*

Reading	Surface	Depth	Temperature, °C.
1	Top	12 inches	50.
2	do	do	66.
3	do	48 inches	36.
4	Side	12 inches	67.
5	do	6 inches	77.
6	do	do	82.
7	do	do	80.
8	do	12 inches	78.
9	do	6 inches	76 near smoking straw.
10	do	Surface	87 in smoking straw.
11	do	do	132 in glowing straw.
12		Removed from pile	167 in glowing straw.

The temperature of the opposite or east side of the pile was also excessively high, though no fire had yet appeared. This side was

FIG. 3.—Site of third outbreak of fire; A, location of hottest belt; east side

watched carefully. The following afternoon, September 10, fire broke out on this side, appearing first, as the others had, at from 5 to 10 feet above the ground.

When this had been put out an inspection of the stack revealed so many hot areas along the edge that the entire side was removed under frequent sprinkling with water. (Fig. 3.)

The mass was packed so firmly that progress was slow and fire broke out twice before the work was completed. In one of the hottest sections (fig. 3, A) a forkful of material was removed and spread out upon the ground. The straw was hot and steaming. About one minute later the steam had changed to smoke, which increased in density until after about three minutes the material glowed a fiery red. Exactly the same conditions appeared in the stack adjacent to the spot from which the material had been removed.

Temperature readings of the glowing coals and adjacent materials (though those of the former are probably only approximate) gave the following results:

	° C.
Temperature of glowing straw removed from pile	167
Temperature of glowing straw remaining in pile	132
Temperature in stack 3 inches from glowing coals	80

The fire smoldered a few minutes, then went out. It is significant that the temperature of material only a few inches from the glowing coals was not above 80° C. Moisture determinations showed that material in the stack adjacent to the point where the red glow had appeared contained only 3.7 per cent of water, whereas material 2

FIG. 4.—Normal and charred material

feet away, apparently just as badly scorched and brittle, contained 30.2 per cent. Material from the top of the stack, which was hot but showed no signs of scorching, contained 66 per cent moisture.

Figure 4 shows the contrast between material which has undergone excessive heating without firing and normal material from the surface of the pile. The charred material was very dry and brittle.

As the heating mass of material offered excellent opportunities for study, the following day further data were obtained. In general, the mass had cooled considerably, though a few areas were still hot.

Since "spontaneous" ignition has been known to take place apparently only as the result of rapid oxidation, it was assumed that further temperature rise would be produced in the manure pile on the introduction of air, or, preferably, pure oxygen. A brass tube and the thermocouple were introduced simultaneously into the center of an area having a temperature of 82.5° C. At 11.25 a. m. oxygen was introduced from a pressure tank, and changes in temperature were carefully noted. The temperature immediately began to rise rapidly and continued to increase for one-half hour; the rate of oxygen flow was occasionally increased. (Table 2.) At 12 m. the temperature had reached 109° C., an increase of 26.5° in 30 minutes. Further aeration resulted in a slow cooling to 107° at 1 p. m. As the heating

material was inclosed within the mass, the estimation of the oxygen requirements was purely conjectural. Thus it is not surprising if the oxygen rate most favorable for heat production was not maintained and only limited heating allowed to take place.

TABLE 2.—*Temperatures obtained by introducing oxygen into hot area of manure pile (2 feet beneath side surface)*

Reading	Time	Temperature	Oxygen flow	Reading	Time	Temperature	Oxygen flow
		°C.				°C.	
1	11.24 a. m.	82.5		8	11.43 a. m.	106.0	
2	11.25 a. m.	84.0	Begun.	9	11.45 a. m.	107.0	Increased.
3	11.30 a. m.	94.0		10	11.50 a. m.	108.0	
4	11.34 a. m.	100.0	Increased.	11	12.00 m.	109.0	
5	11.36 a. m.	103.0		12	12.15 p. m.	108.5	
6	11.38 a. m.	104.0		13	1.00 p. m.	107.0	
7	11.41 a. m.	105.0					

SUMMARY

A pile of heating stable manure was observed to ignite "spontaneously." When exposed to the air, charred straw glowed a fiery red. Oxygen aeration of a small section of the heating material produced a rapid increase in temperature of 26.5° C. in 30 minutes.

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